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STUDIES ON THE LIFE HISTORY OF *LINSTOWIELLA SZIDATI* (ANDERSON) (TREMATODA: STRIGEATOIDEA: CYATHOCOTYLIDAE)[1]

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INTRODUCTION AND HISTORICAL REVIEW

In a preliminary abstract, one of us (Anderson, 1945) reported the determination of the life history of *Cercaria szidati* Anderson, 1944. The present paper includes the experimental demonstration of the life cycle, the morphology of the various stages, and a supplement to the earlier description of the cercaria, particularly in respect to the development of the excretory system.

The adult has proved to be an undescribed species of *Linstowiella.* In accordance with the Rules of Nomenclature, its correct name is *Linstowiella szidati* (Anderson, 1944) *comb. nov.,* since the larva, *Cercaria szidati,* was named before the adult was known. To follow custom, the larva would now be referred to as *Cercaria Linstowiellae szidati* or, more simply and preferably, the cercaria of *Linstowiella szidati.*

The cercaria of *L. szidati* is a furcocercous larva of the type Sewell (1922) placed in Group 3 of his classification. His subdivision of the group and subsequent modifications thereof have been reviewed by Cable (1938) who redefined and simplified the classification to a considerable extent. Further simplification was proposed by Anderson (1944). Cable's list of described species may be brought up to date by the addition of *Cercaria theodoxia* Porter, 1938, the cercaria of *Paracoenogonimus ovatus* Katsurada, 1914, as described by Komiya (1939), *Cercaria tatei* Johnston and Angel, 1940, *Cercaria notopalae* Johnston and Beckwith, 1945, an unnamed form described by Maxon and Pequegnat (1949), and the present species.

This study makes a total of seven life histories more or less completely traced for members of the group. Azim (1933) observed that *Cercaria vivax* Sonsino, 1892, encysted in small fishes and he obtained the adult stage by feeding infected fish to dogs and cats. He identified the adult as *Prohemistomum vivax,* a species which Odhner had described from the Egyptian kite and named *Prohemistomum spinulosum.* Szidat (1933) traced the life cycle of *Distomum viviparae,* described by von Linstow from metacercariae in *Vivipara vivipara,* and erected the genus *Linstowiella* with *L. viviparae* as type. The cercariae of this species encysted in

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the molluscan host and immature adults were recovered from rats fed infected snails. Szidat also described two related cercariae and later (1936) reported the life history of one, *Cercaria curonensis,* which he found to be the larva of an adult for which he erected the genus *Cyathocotyloides.* The cercariae of *C. curonensis* encysted in fish and adults were obtained by feeding infected fish to the domestic duck. The experimental determination of the life cycle of *Cyathocotyle gravieri* was reported very briefly by Mathias (1935). He infected carp and minnows with furcocercariae from *Bythinia tentaculata* and recovered adults from ducks fed metacercariae in fish. Mathias' description of the cercaria is incomplete. However, he probably was dealing with a member of the "Vivax" group since the undeveloped ventral sucker and the distinctive intestinal ceca as he described them, are both characteristic of that group. Komiya (1939) described the life cycle of *Paracoenogonimus ovatus* Katsurada, 1914. The cercariae develop in *Vivipara vivipara* and encyst in fish. Adult worms were recovered from mice and rats, and dogs fed metacercariae passed eggs in the feces. In addition to the above, the life cycles of several other cyathocotylids have been traced only to the extent of determining the metacercaria (see Dubois, 1938). In but one of these, *Szidatia joyeuxi,* is there any indication as to what the cercaria might be. This species was first described as the metacercaria from frogs and fish at Gafsa, Tunisia, and later, in frogs at Biskra, Algiers. Joyeux and Baer (1934) fed parasitized frogs to a snake from another region (Italy) and recovered adult worms similar, except for degree of maturity, to a species occurring naturally in viperine snakes at Gafsa. They suggested that *Cercaria vivax* Langeron, 1924, *nec* Sonsino, a species common in the locality, might be the larval stage, but gave no experimental evidence.

METHODS

Methods used in the description of the cercaria (Anderson, 1944) were employed in further study of larvae emerging from the snail host, *Campeloma rufum.* It was found that infected snails would live for several months if held at ice box temperature and that sporocysts obtained by cracking them could be kept alive in the refrigerator for 10–14 days if placed in 0.1 to 0.3% NaCl solution. Thus, abundant material for observing the development of the cercarial excretory system was available over an extended period.

Experimental infection of second intermediate hosts was accomplished by exposing them to snails shedding cercariae in an aquarium. Metacercariae from both naturally and experimentally infected intermediate hosts were studied and compared. In efforts to digest the metacercariae from the cysts, both trypsin and pepsin were used under varying conditions. Potential definitive hosts were exposed to infection either by spontaneous or forced feeding of infected intermediate host. Whole mounts of the various stages were stained with hematein and Mayer's paracarmine.

OBSERVATIONS

Demonstration of the life cycle

Investigation of the life cycle of *Linstowiella szidati* began with attempts to determine the second intermediate host and thus obtain metacercariae. Both infected and uninfected *Campeloma rufum* were exposed for prolonged periods to cercariae

of *L. szidati* since this species is very similar to the larva of *L. viviparae* which penetrates and encysts in the molluscan host. Observation of snails during exposure showed no tendency of cercariae to penetrate them nor were metacercariae ever recovered when the snails were cracked and examined later. The same was true of all other common species of mollusks occurring in the river from which the infected snails were collected.

After it was found that a mollusk evidently could not serve as the second intermediate host of *L. szidati,* experiments were carried out testing various species of fish including several sunfish, the bluegill, catfish, and several species of minnows. Of these, it was found that the cercariae would penetrate and encyst in numbers only in the river shiner, *Notropis cornutus* subsp., although a few cysts were recovered from bluegills after prolonged exposure to large numbers of larvae. The minnow is very abundant in the stream from which infected snails were collected. Because naturally infected river shiners were found and in view of the ease with which they acquired massive experimental infections, it seems evident that this minnow serves as the natural second intermediate host of *L. szidati.*

To obtain metacercariae for experimental use, minnows were placed in an aquarium with two to four infected snails for 48 hours. A longer exposure resulted in such heavy infections that all the fish died, and even a 48-hour exposure caused the death of about 50 per cent.

To observe the penetrating cercariae, minnows were confined in a small open-top wire cage to restrict movement. The cage was then placed in a small dish of water containing cercariae and the preparation observed with a dissecting microscope. The larvae seemed to be stimulated by water currents and swam toward the minnow. On contact, they immediately worked their way into the skin, going between the scales. When the larva had penetrated about half its body length, the tail separated and floated free with the furcae coiled.

Metacercariae became fully developed at about three weeks of age and presumably were infective for the definitive host. Attempts to determine this host were carried out in a series of feeding experiments. First, each of three white rats were force-fed by stomach tube approximately one hundred cysts in small portions of infected muscle tissue. After feeding, the rats were killed at intervals of four, five, and six days respectively and the intestinal tract examined, but no worms were found. Similar feeding experiments were performed with white mice; three were fed metacercariae and when killed one, two, and three days respectively after receiving cysts, all were found to be negative.

In another series of experiments, three mallard ducks were force-fed approximately a hundred cysts each and killed at intervals of three, five, and twelve hours after feeding. Negative results were obtained in the two longer periods, but some dead and partially disintegrated metacercariae were found in the upper region of the small intestine of the duck killed three hours after feeding. Next, three two-day old chicks were fed metacercariae and examined 12, 24, and 48 hours later. No trace of the cysts could be found.

Ferguson (1936) had reported the experimental infection of chicks with the strigeid, *Posthodiplostomum minimum,* a parasite of the heron. He was able to infect young chicks that had never received food, but not those that had been fed. In view of his observation, it was decided to try newly-hatched chicks as an experi-

mental host of *L. szidati.* In the first series, five chicks were fed metacercariae and killed later for examination. From one killed after five hours, 21 immature flukes were recovered from the middle part of the small intestine. On the third day after feeding, all of the remaining four chicks began to pass eggs in the feces. One killed the following day harbored 14 sexually mature worms. All were in the middle region of the small intestine. The three remaining chicks were killed on the seventh, tenth, and fifteenth days respectively but no worms were recovered, although they were known to have passed eggs previously.

Three additional series of four to six newly-hatched baby chicks each were fed metacercariae of *L. szidati* in precisely the same manner as in the series which had yielded positive results. However, no worms were recovered from any of them when killed at five-hour to 14-day intervals later.

Since it had been determined that the worms may attain sexual maturity in three days, a final feeding experiment with baby chicks was performed after termination of the work with herons reported below. Four chicks that had just hatched were fed numerous metacercariae in minnows infected four months previously. Thereafter, the birds received water only and on the third day, eggs were found in the feces of all of them. One was passing a large number, the others, only a few. All were killed and examined that day. No worms were found in one but the other three harbored two, four, and 85, the last number being in the bird passing the most eggs. Since all four chicks were the same breed and were handled in as nearly identical manner as possible, the only explanation for the difference in the number of worms recovered as well as the negative results of previous experiments with unfed chicks would seem to be a matter of individual differences in susceptibility to infection.

Although the identity of the adult worm had been established and the egg size determined from the successful experiments with the chick, this host was too variable in susceptibility to infection and lost the worms too rapidly to serve as a dependable source of eggs for further study of the life cycle. Since a piscivorous bird was indicated as the natural definitive host, two nestlings of the heron, *Ardea herodias,* were obtained. Fecal examination revealed that both were heavily infected with trematodes whose eggs could not be distinguished with certainty from those of *L. szidati.* For this reason, an attempt was made to remove the flukes present. First, the mouth and throat of each bird was swabbed with concentrated gentian violet solution which was entirely effective against *Clinostomum marginatum,* many of which were present. Then hexylresorcinol was administered in an attempt to remove intestinal helminths. This was not followed by an appreciable drop in the number of eggs in the feces. Finally, enteric-coated gentian violet pills were administered to one bird at the rate of one-third the usual adult dosage for *Enterobius vermicularis* in man. This proved to be toxic to the bird and apparently was the cause of its death at the end of the fifth day. Post mortem examination was negative for helminths, indicating that the gentian violet had been effective. The remaining bird was then treated at the rate of one half the dosage received by the first and watched closely for toxic symptoms. It became sick and refused food. Treatment was discontinued and recovery was rapid. The egg count in its feces fell to a very low value, but an occasional one was still present. When a second treatment following a rest period failed to eliminate eggs completely from the feces, the bird was fed a large number

of *L. szidati* metacercariae. The egg count rose abruptly at the end of the second day, continued at a high level for several days and then dropped to a somewhat higher level than before the bird was fed metacercariae. Three subsequent feedings, each given after the egg level had dropped, yielded similar peaks of egg production but each was lower than the preceding one and fell to a lower level. After a slight peak following the fourth feeding, the egg count dropped until only an occasional egg could be found in the feces.

It was finally decided to give a fifth feeding and kill the bird to get adult worms for comparison with those obtained from the chick. On examination three days after this feeding, the bird was negative for *L. szidati* but was found to harbor a single strigeid which had apparently been the source of the few eggs that had been observed from the beginning. Evidently the repeated infection of this bird with *L. szidati* had resulted in complete immunity to this species.

The natural definitive host of *L. szidati* has not been determined. Although the green heron, *Butorides virescens virescens,* is common in the locality where snails are infected, a careful examination of six individuals collected there in connection with another study has not revealed the presence of *L. szidati.* Three individuals of *Totanus flavipes,* the lesser yellow legs, also were not infected. There has been no opportunity to examine the kingfisher, *Megaceryle alcyon alcyon,* which may be the natural definitive host. After the discovery in this laboratory of a species of *Cyathocotyloides* in the intestine of the channel catfish, *Ictalurus punctatus,* three individuals of this species were fed metacercariae of *L. szidati.* Subsequent examination yielded only large numbers of immature bucephalids which were also present in the infected minnows which the fish received. While it is possible that some other fish or perhaps a reptile may serve as the natural definitive host of *L. szidati,* the experimental infection of the chick and blue heron suggests that the natural host is not a poikilothermic animal.

Description of Stages in the Life History

The Adult (Figs. 3–5)

Specific diagnosis. (Measurements are in mm and taken from whole mounts of specimens killed under light coverglass pressure.) With the characters of the genus *Linstowiella* as emended below. Body length 0.416–0.565 (average, 0.478); maximum width 0.312–0.371 (average, 0.344). Oral sucker 0.028–0.057 (average 0.052) in diameter, wider than long in contracted specimens; pharynx 0.037–0.041 (average 0.039) in length; esophagus very short, ceca long, extending to level of posterior testis. Tribocytic organ weakly developed, protruding but never observed everted. Body spinose, spines prominent on ventral surface to edge of opening in tribocytic organ, becoming less numerous toward posterior end. Testes elongate transversely, situated in tandem on left side of body, measuring 0.120×0.07 to 0.162×0.120 (average 0.145×0.091); cirrus sac present. Ovary on right side opposite anterior testis, small, averaging 0.045×0.060, often obscured by vitelline follicles and difficult to see. Vitelline cells large, usually not quite meeting anteriorly but with a posterior confluence, probably of cells in the vitelline ducts. Eggs one to three in the uterus at one time and measuring from 0.108×0.076 to 0.120×0.082.

Hosts: (experimental) *Gallus domesticus* and *Ardea herodias*

Locality: Tippecanoe River near Lafayette, Indiana, U. S. A.

Type specimens: Holotype No. 46397, Helminthological Collection, U. S. National Museum.

When the host's intestine is opened, the worms are easily removed, the adhesive organs appearing to be rather weak in this species. It is very active and remains so for several hours in saline at room temperature. The ventral concavity, although pronounced (Fig. 3), is not as deep as in such other genera as

Cyathocotyloides. The caudal papilla corresponding to the hind-body of strigeids is small and usually not evident in whole mounts. The large lateral nerve trunks are prominent anterior to the vitelline follicles. It was not possible to determine the excretory pattern of the adult; only a few flame cells were seen for details of the pattern were obscured by glandular structures and the complex reserve excretory network extending throughout the body.

In his diagnosis of the genus *Linstowiella,* Szidat (1936) included as one character the apparent reduction of the male genitalia to a seminal vesicle free in the parenchyma. Such is not the case in *L. szidati,* which possesses an unmistakable cirrus sac (Fig. 4). Because of the small size of the worm and the fact that the cirrus sac is not distinct in stained preparations, it was necessary to study living material at considerable length to establish this point. It was not until prostate cells and their ducts were detected that it could be ascertained that the structure was not simply a fairly thick-walled seminal vesicle. These gland cells are extremely delicate and finely granular and their ducts can be seen as a single row of granules extending posteriorly and turning to enter the sperm passage. Where they do so, there may be seen distinct masses of granular material within the passage; whether these are masses of prostatic secretion or merely the enlarged tips of the ducts cannot be stated. The portion of the sperm duct into which the prostatic ducts open is not differentiated to form a distinct pars prostatica although that region may be slightly enlarged. In one specimen studied alive with the oil immersion lens under considerable coverglass pressure, the cirrus was seen protruding from the genital pore. It was very slender and unarmed.

The Egg

The eggs of *L. szidati* are very large for the size of the worm. They are oval-round, light yellow, with one end somewhat pointed and bearing a small operculum. There is nothing characteristic either as to size or appearance that distinguishes the eggs of this species from those of many other holostomes.

Eggs recovered from the feces of experimentally infected birds were incubated in small dishes of aerated water. The first cleavage, which is unequal, is usually completed in four hours. As development proceeds, yolk begins to coalesce, forming large globules surrounding the embryo. By the end of the third day, these globules conceal the embryo almost completely and further development is not apparent until the fifth day when the double eye-spot appears. The yolk is less noticeable thereafter, and at 8 to 11 days, two pairs of flame cells can be distinguished within the embryo which elongates and becomes motile. Hatching occurs in 11 to 18 days, depending on temperature.

The Miracidium

Numerous attempts were made to obtain miracidia by incubating eggs in dishes of aerated water. The larvae developed normally for several days, often until the eye-spot and flame cells could be seen through the egg shell, but most of them failed to hatch. Of the few that did, some were studied alive while the remainder were either fixed and stained with hematein or used in an unsuccessful attempt to delineate the epidermal cells by silver impregnation.

Stained specimens measure about 0.135 long and 0.035 in maximum width. The terebratorium may be retracted and in that state measures 0.008 from base to tip.

The double eye-spot is 0.006 long and situated at approximately one-third the body length from the anterior end; the pigment cups are distinctly separated. The middle of the body is occupied by the large ganglionic mass of numerous small nuclei. Just posterior to this mass are a few single germ cells with large nuclei and distinct cytoplasmic prolongations. Posterior to these was seen in both living and stained miracidia, a spheroid mass containing in one specimen four large nuclei and apparently enclosed in an investing membrane. This structure is interpreted as a germ mass which probably serves as a center of germinal multiplication in the mother sporocyst. The intact nature of this mass indicates that the single germ cells anterior to it were not derived from it; instead, it seems more likely that they also are destined to form similar masses after the miracidium penetrates the snail and becomes the mother sporocyst.

The excretory pores of the miracidium are dorsolateral, near the posterior end. Associated with each is a small vesicle and a pyriform structure resembling what has been called an excretory cell in other species. Each main excretory tubule extends anteriorly in the manner shown in Fig. 1. Near the eye-spot level, it turns posteriorly and then laterally at the ganglionic level where it receives the capillaries of an anterior and a posterior flame cell. The excretory formula of the miracidium accordingly is 2 (1 + 1), the same as that in other members of the STRIGEATOIDEA.

The Sporocyst

Only sporocysts producing cercariae have been found, and they were mentioned briefly in an earlier paper (Anderson, 1944). Mother sporocysts have been described for other species of "Vivax" cercariae and have been recovered in this laboratory in the routine dissection of snails infected with another species of this type. In the case of *L. szidati,* however, they apparently do not persist after establishment of the daughter sporocyst generation since they could not be found in a large number of infected snails, some of which were not yet shedding cercariae. There were never sufficient miracidia to attempt infections of the mollusk and thus obtain young sporocysts.

The smallest sporocysts observed measured about 0.045×0.01 mm. while those containing fully developed cercariae attained a length of over 0.5 mm. They are sausage-shaped with prominent elevated transverse muscle bands, thus resembling closely the sporocysts described for related species. They are very active, especially at one end which because of its exploratory movements and structure is considered to be the anterior end. There are numerous gland cells in this region which in young sporocysts is often bulbous. The birth pore is slightly posterior to the anterior end and can be seen more clearly in sporocysts of medium size than in large ones. Cercariae have been observed escaping through this pore.

The excretory system of the sporocyst is exceedingly complex and only portions of it could be observed because of the large number of minute refractile granules or droplets in the body wall. The excretory pores are near the posterior end. From each, a canal extends forward, but details of its complex branching could not be determined. It is possible that the basic pattern is similar to that described by Looss (1896) for the sporocyst of *Cercaria vivax.* In that species, each main tubule divided into a long anterior and a very short posterior collecting tubule, each receiving the capillaries of three flame cells. This is the basic pattern seen in the

cercarial embryo of *L. szidati* (Fig. 9). Unless it would be in extremely young sporocysts of this species, however, the excretory pattern is by no means as simple as in the sporocyst of *C. vivax*. Over 40 flame cells have been counted on one side in young sporocysts of *L. szidati,* and it has been determined that they occur three to a group as in the emerging cercaria.

The Cercaria and the Development of Its Excretory System

The morphology and behavior of the cercaria have been described in a previous paper (Anderson, 1944). It should be added that the larva, unlike many furcocercous species, does not swim tail first. While there is some movement of the tail as a whole, locomotion seems to be effected mostly by flexing the tail stem at the point where it is always bent when the cercaria is at rest. This causes a typical jerky swimming movement in an upward direction. Swimming is intermittent and unless disturbed, most of the cercariae hang suspended in the water but not in contact with the surface film. The tail stem straightens only in moribund larvae or when subjected to cover glass pressure or fixatives. A correction of the earlier description is necessary, however, as revealed in the study of the development of the excretory system. Instead of the anterior collecting tubule having two groups of three flame cells and the posterior four groups as described, both tubules have three groups each. The corrected flame cell formula accordingly is $2[(3+3+3)+(3+3+3)]$ with the posterior group of each side in the tail stem as described.

In a critical study of numerous cercarial embryos, there was observed no germ ball with fewer than two pairs of flame cells (Fig. 6). This is in agreement with observations on other species of "Vivax" cercariae (Looss, 1896, and Komiya, 1939), and it seems probable that the cercarial embryo of this group never contains a single pair of flame cells as in many of the PROSOSTOMATA and certain other furcocercous species (Hussey, 1941).

The youngest embryo showing flame cells is almost spherical with two excretory tubules extending about two-thirds of the length of the embryo and arching slightly towards the center. From the anterior end of each of these, a short narrow tubule extends laterally and receives the capillaries of an anterior and a posterior flame cell.

As the embryo grows, it begins to elongate and the excretory tubules follow suit. The two main tubules come closer together and their anterior ends become enlarged and ciliated (Fig. 7). At this stage, there is no change in the flame cell pattern.

The embryo continues to elongate (Fig. 8) and becomes truncate. A slight incurving of each side indicates the beginning of the tail. At that level, each main excretory tubule sends off a short median branch and then swings laterally so that the two main tubules become more widely separated than in the preceding stage. Their anterior tips remain swollen and a short branch arises from the median side of each. The tips are ciliated but the branch is not. Each of the flame cells present in the preceding stage is now represented by a pair.

With continued development (Fig. 9), the median branches of the main tubules grow anteriorly, converge without fusing, and then separate. The paired excretory pores are now at the tips of the furcae which are just becoming evident. A flame cell is added to each of the pre-existing groups, making a total of four groups of three flame cells each.

As development and growth of the cercarial embryo continue, the furcae and the tail stem become more clearly delineated (Fig. 10). The median tubules elongate, reaching the level of the lateral tubules, and grow towards median projections of the lateral tubules. The median tubules are contiguous anteriorly, but not yet fused. An expansion of each common tubule just posterior to the junction of the median and lateral ascending tubules forms the first indication of the excretory vesicle. At this stage, the posterior-most flame cell capillary on each side has lengthened so that the flame cell lies in the tail stem region which now is distinct from the body proper.

As the cercaria develops further, the cilia in the anterior end of each lateral tubule become less evident and changes in the excretory system consist largely of fusions of the various tubules (Fig. 11). The median ones unite along the extent to which they were contiguous in the preceding figure and their tips fuse with the projections from the lateral tubules to become the cross commissure of the emerging cercaria. The tips of the lateral tubules remain as projections. The enlargements of the common or main tubules grow medially towards one another and the two tubules fuse for a part of their extent through the tail stem. The flame cell formula remains unchanged although the collecting tubules become more convoluted and arise from the median side of the lateral ascending trunks.

With further development (Fig. 12), the excretory pattern becomes essentially complete, although the cercaria is only about two-thirds its ultimate size. The enlargements of the main tubules at the junction of the body and tail meet and fuse to form the excretory vesicle. The Island of Cort appears between this and the point at which the tubules unite in the tail stem. The common caudal tubule increases in length by continued fusion of the original tubules and elongation of the tail stem, near the posterior end of which the original tubules separate to enter the furcae and open near their tips. Each flame cell of the six pairs in Fig. 11 is now represented by a group of three flame cells as in the emerging cercaria. There is no trace of the cilia observed in the lateral tubules in the earlier stages.

The Metacercaria (Fig. 2)

Metacercariae may occur throughout the body musculature of the minnow but are especially numerous in the caudal region. Since they are never found in the skin and do not become pigmented, their presence would not be revealed by superficial examination of the skin. Because of their almost spherical shape, they are readily differentiated from the more elongate cysts of gasterostomes which were always abundant in the minnows used. There are two cyst walls, an outer which is fragile and easily ruptured and an inner which is extremely tough and difficult to remove without injury to the worm. Peptic and tryptic digests at 37.5 and 40.0° C. released worms but killed them in the process. With lower concentrations of enzymes or lower temperatures, the inner cyst wall failed to rupture.

The metacercaria is at first round and has but one cyst membrane. At two days of age, cysts average 0.18×0.15 and the worms are so vacuolated that internal structures cannot be seen. By the fourth day, the average size increases to 0.23×0.17, the second cyst wall is formed, and opaque granules appear scattered throughout the body of the worm. At 12 days, the average size of the cyst is 0.32×0.24 and the oral sucker and tribocytic organ become evident. The reserve excretory net-

work is delineated by granules and becomes conspicuous. At 20–24 days, the cysts attain their maximum size, averaging 0.4 × 0.3, and the metacercariae are infective as determined by feeding experiments.

DISCUSSION

Linstowiella szidati is the second species of the genus and the first from North America. The other is *L. viviparae* (von Linstow) Szidat, 1933, which was more completely described by Szidat (1936). In his brief description of *Mesostephanus longisaccus,* Chandler (1950) mentioned the resemblance of that species to *Linstowiella* but chose to allocate it to the genus *Mesostephanus* because of the presence of a large cirrus sac and a distinct caudal appendage. In lacking a ventral sucker, *M. longisaccus* is in agreement with *Linstowiella* and the present study shows that a cirrus sac may be present in that genus. However, *M. longisaccus* is more like *Mesostephanus* than *Linstowiella* in the size of the cirrus sac, the relative extent of the vitelline follicles, and the presence of a distinct caudal appendage. Although this appendage is present in *Linstowiella,* Szidat (1936) described it as being apparent only momentarily in living specimens of *L. viviparae.* While this structure is plainly visible in living material of *L. szidati,* it cannot be seen in any of the writers' stained preparations whether fixed with or without pressure after chilling or shaking to relax the worms. Prof. Chandler has kindly provided his specimens of *M. longisaccus* and the type has been made available to the writers for examination. These specimens, unlike *Linstowiella,* show a distinct caudal appendage. It is mostly for this reason that allocation of *M. longisaccus* to *Linstowiella* does not seem justified at present. In fact, such a proposal would necessitate reducing *Mesostephanus* to synonomy with *Linstowiella* which may be desirable when additional species are discovered. For the time being, however, it is suggested that the two genera remain distinct and that the genus *Linstowiella* be emended as follows to include information provided by the present study:

Genus *Linstowiella* Szidat, 1933, emend.

With the characters of the Family CYATHOCOTYLIDAE, subfamily PROHEMISTOMINAE (see Dubois, 1938). Body small, and undivided but with short cone-shaped caudal protuberance visible in living specimens. Dorsal surface convex, ventral concave with protruding tribocytic organ which may be fairly large or weakly developed; ventral sucker absent. Opening of tribocytic organ elongate with crenate margin. Vitelline follicles large, extending through the posterior one-half to two-thirds the body length; arranged in a corona encircling the tribocytic organ. Testes situated one behind the other, to the left of the median line; ovary right, opposite or slightly posterior to anterior testis. Cirrus sac present or apparently reduced (?) to a simple claviform seminal vesicle free in the parenchyma. Genital pore posterior, at tip of caudal protuberance. Adults in the intestine of birds and mammals. Cercaria of the "Vivax" type without caudal finfolds, develop in simple, elongate sporocysts in prosobranch mollusks; metacercariae in mollusks and fishes.

Includes:

Type species, *Linstowiella viviparae* (von Linstow) Szidat, 1933. Syn. *Monostomum viviparae* von Linstow, 1877. *Linstowiella szidati* (Anderson) comb. nov. Syn. *Cercaria szidati* Anderson, 1944.

In distinguishing the above species the slightly larger size of *L. szidati* would seem to be significant, especially since it is known only from three-day-old infections of chicks which certainly are not natural hosts and in which the species would not be expected to attain its maximum size. The difference in number of eggs in the

uterus, one to three in *L. szidati* and one in *L. viviparae,* might be significant only were it known that Szidat's material of the latter species was fixed immediately after sacrifice of the host; it was observed in the present study that after specimens of *L. szidati* were placed in saline, eggs were extruded, often until the uterus was empty.

The most convincing and significant evidence that *L. viviparae* and *L. szidati* are distinct species is provided by their life histories, and especially by the fact that one utilizes the molluscan host as the second intermediate host while the other encysts in minnows. The cercariae of the two species are very different in respect to size and excretory pattern, the larva of *L. szidati* being much the larger of the two and having a larger number of flame cells in the body. The number of flame cells per group in the body cannot be determined from Szidat's figure of the cercaria of *L. viviparae,* but the total number, nine on each side, suggests that they are arranged in groups of three's as in the caudal groups. If that is true, then the excretory formula for his species would be $2\ [(3+3)+(3+3)]$ with the last group in the tail stem. Szidat's representation of the anteriormost flame cell capillary joining the main lateral tubule probably is incorrect and, although he does not show it, the posterior collecting tubule probably continues into the tail stem to receive the capillaries of the flame cells there.

Although a number of furcocercariae of the "Vivax" type has been reported, the development of the excretory system of only two species has been hitherto described, *C. vivax* Sonsino by Looss, (1896) and the cercaria of *Paracoenogonimus ovatus* by Komiya (1939). At the time the embryological observations of the present study were made, Komiya's work was unknown to the writers. When his paper became available, it was interesting to find that, step by step, his account corresponded almost exactly with observations on the cercaria of *L. szidati.* The only noteworthy difference was that Komiya did not mention ciliated enlargements at the anterior end of the lateral ascending excretory canals in early stages. Looss' account, on the other hand, gives an entirely different interpretation of the origin of the median excretory canals, one in fact, which makes it difficult to understand how the ultimate pattern could have been attained. In the youngest stage, he described the pair of main tubules as being Y-shaped, each arm terminating in what appeared to be flame cells. This stage was essentially the same as the youngest ones observed by Komiya and the writers (Fig. 6). Looss then described fusion of the median arms of the tubules to form the single median tubule instead of its formation by later outgrowths of the main tubules and their fusion as observed by Komiya and the authors. Looss' account of the formation of the anterior commissure also is not in agreement with present observations. His description of the "germigene" in the sporocyst is one of the most complete to be found in the literature. Such structures also occur in the sporocysts of *L. szidati* and appear to function as persistent centers of germinal multiplication.

In his description of *Cercaria Indica XV,* Sewell (1922) stated, "The development of this fork-tailed cercaria is extremely remarkable in that the sporocysts have the power of producing not only cercariae but miracidia. . . ." Although his description of these miracidia is explicit, neither this statement nor his figures definitely indicate whether miracidia are produced by mother or daughter sporocysts, both of which he observed in this species. Since mother sporocysts produce sporocysts, the

implication is that miracidia were observed in the sporocyst generation which also gives rise to cercariae. A special search for miracidia of sporocyst origin was made in the present study but without success. In his detailed study of the development of *Cercaria vivax,* Looss (1896) did not describe anything that could possibly be interpreted as miracidia in the sporocysts. It may be significant that Sewell observed several "parent sporocysts" in a single snail whereas Looss found but one and the writers were unable to find this stage. The presence of several mother sporocysts of *C. indica XV* in a single snail may have been due to (1) multiple infection by miracidia hatched from eggs on the outside; (2) the intercalation of another sporocyst generation between the miracidium-mother sporocyst and the cercaria-producing generation; or (3) to a phase of sporocyst production by the generation which may also produce cercariae. The situation in this species, however, offers still another explanation, viz., the development of sporocyst-produced miracidia into mother sporocysts. The unique situation in *C. indica XV* calls to mind Woodhead's (1931) observation of ciliated "pro-rediae" in the sporocyst of a bucephalid. The interpretation of these findings from the phylogenetic standpoint is difficult. In aspects of the digenetic trematode life cycle and in the Aspidogastrea, many helminthologists see the ancestral Digenea as monogenetic parasites of archaic mollusks. As such, they would have attained sexual maturity in the mollusk, producing eggs from which miracidia hatched. The production of miracidia within the molluscan host of *C. indica XV* might therefore be interpreted as a primitive characteristic. However, until this phenomenon is confirmed in other species and the manner in which the miracidia develop is understood, interpretation of Sewell's observations as either the retention of a primitive phase or a coenogenetic intercalation in the life cycle would be mere conjecture. For reasons given below, the writers regard the cyathocotylids as the most primitive of the holostomes. If, however, the production of miracidia by sporocysts is a primitive character, it might not be expected to occur in species of *Linstowiella,* for this genus does not seem to us to be a primitive one among the cyathocotylids.

The absence of a ventral sucker in *Linstowiella* raises the old question of monostome versus distome ancestry in the Digenea. However, there now seems sufficient evidence from other studies to expect the sporadic occurrence of monostome genera in groups that are fundamentally distomatous. Nor is the evidence confined to adhesive organs. The absence of a pharynx in the genus *Apharyngostrigea* in a group that is otherwise pharyngeate is an excellent example. Others could be cited concerning terminal genitalia and modifications of the digestive system including secondary anal openings. For these reasons, the absence of a ventral sucker in *Linstowiella* is believed to be secondary even though there is no indication of its primordium in the embryology of the cercaria.

Although their cercariae are more complex than other strigeoid larvae, the Cyathocotylidae are believed to be more primitive than are the Strigeidae. This belief is based on host-parasite relationships and the morphology of the cercaria and adult. Cyathocotylid larvae, i.e., cercariae of the "Vivax" type, develop in prosobranch mollusks which are more primitive than the pulmonate snails which serve as the predominate intermediate host group of other furcocercariae. In contrast to most trematodes and many strigeids in particular, there is relatively little growth between the cercarial and adult stages of the Cyathocotylidae. For this reason,

the complex structure of the cercaria, especially in regard to the excretory system and the well developed intestine, is interpreted as merely a nearer approach to the adult condition than is true of strigeid cercariae. This might be expected in primitive forms if the mollusk was ancestrally the only host in the cycle, but as in the case of sporocysts producing miracidia, the attainment of an advanced degree of development by cercariae is subject to interpretation as either a primitive or coenogenetic characteristic, in this case, a tendency toward progenetic or paedogenetic development secondarily acquired.

The adult cyathocotylid shows certain indications of being more primitive than are the other strigeoids. In the first place, the cyathocotylids are mostly very small species and the caudal appendage of most of them is not developed to the extent that its homolog, the hind body, is in other strigeoids. The presence of a cirrus sac and simple genital atrium and the absence of accessory suckers seem to be more primitive than the highly modified terminal genitalia and complex fore body to be found among other strigeoids. In these respects, the cyathocotylids are at least more like other trematodes.

SUMMARY

Cercaria szidati (Anderson, 1944) develops in sporocysts in *Campeloma rufum* collected from the Tippecanoe River near Lafayette, Indiana. It encysts in *Notropis cornutus* subsp. and adults of a hitherto undescribed species of *Linstowiella* were recovered from the small intestine of chicks three days after they were fed metacercariae. Various stages in the life history are described including the development of the excretory system in the cercaria. The genus *Linstowiella* is redefined to include *L. szidati* comb. nov.

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EXPLANATION OF PLATE I

(All figures concern *Linstowiella szidati*)

FIG. 1. Miracidium. Combined free-hand drawing from living stained specimens.
FIG. 2. Photograph of a 21-day old metacercaria.
FIG. 3. Free-hand drawing of a living specimen in ventro-lateral aspect.
FIG. 4. Free-hand drawing of cirrus sac in living specimen.
FIG. 5. Adult drawn by microprojection of holotype specimen.

EXPLANATION OF PLATE II

(All figures concern *Linstowiella szidati*)

FIGS. 6–12. Successive stages in the development of the excretory system. All to same scale, free-hand from living material.
FIG. 13. The cercaria showing intestine on one side and excretory pattern on the other.

PLATE I

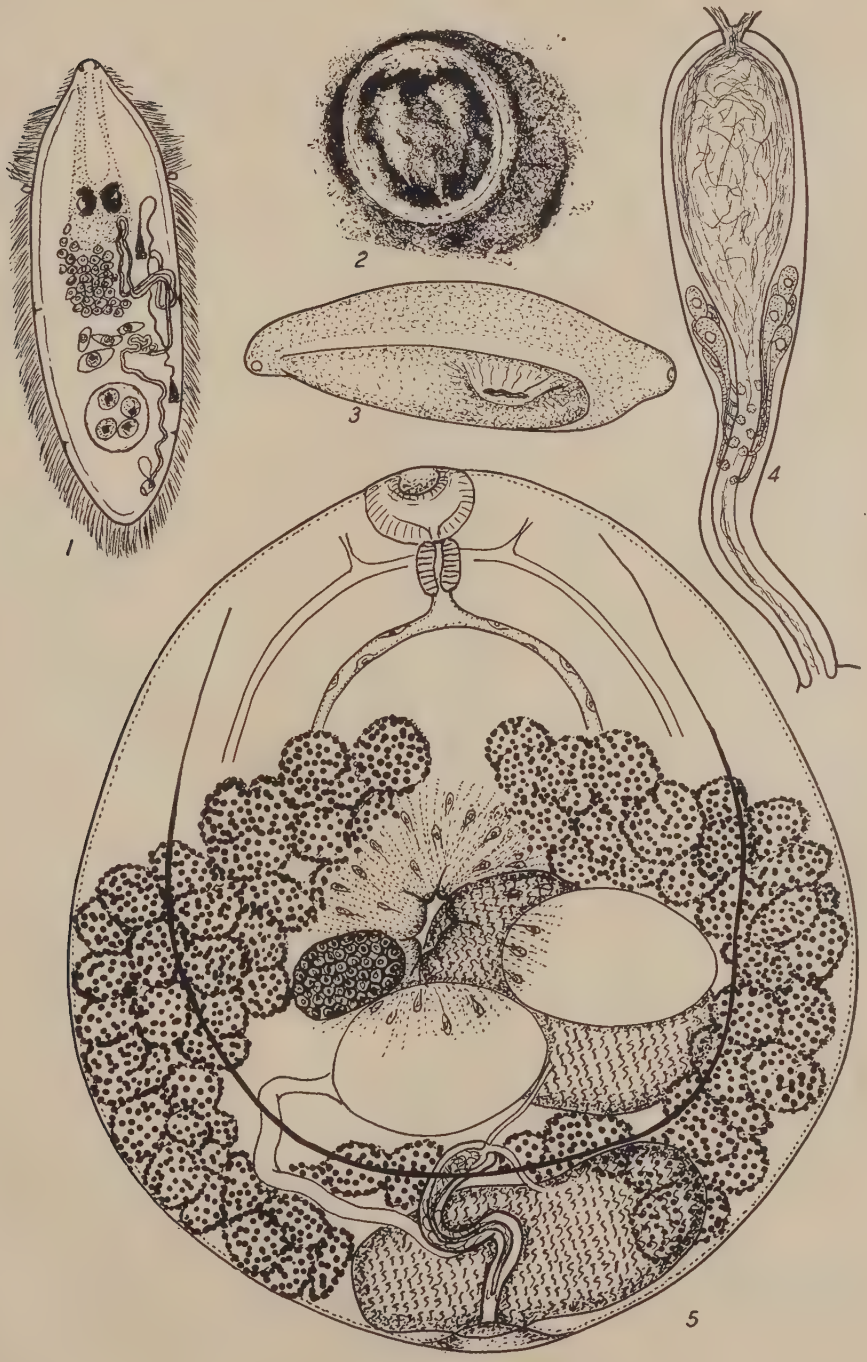
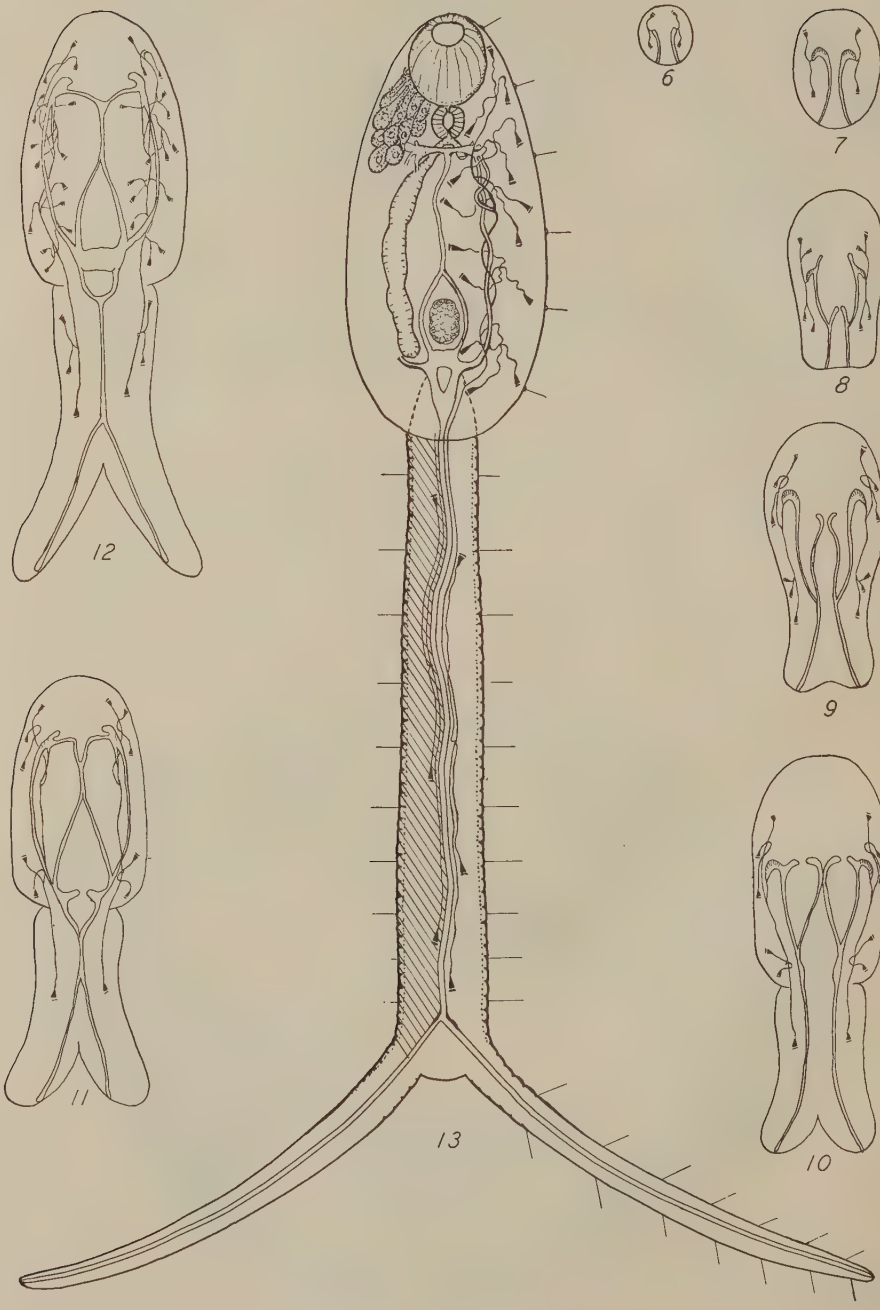


PLATE II



DESCRIPTION OF THE MALE OF THE PINWORM, *SKRJABINEMA PARVA* DIKMANS, 1942 (NEMATODA: OXYUROIDEA) FROM DEER, WITH AN EMENDATION OF THE GENUS

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Six species of the genus *Skrjabinema* are known at the present time. The males, which appear to be much rarer than the females, are known for 3 species. They are *S. ovis* Skrjabin, 1915, *S. rupicaprae* Böhm and Gebauer, 1930, and *S. oreamni* Swales, 1934. Females only are known for *S. alata* Mönnig, 1932, *S. africana* Mönnig, 1932, and *S. parva* Dikmans, 1942.

Dikmans (1942) described *S. parva* from female specimens collected from deer, presumably mule deer, *Odocoileus hemionus* (Rafinesque, 1817), at Boise, Idaho. In addition to the description, he included a review of the literature on the genus and prepared a table of measurements of the females.

During the course of studies on parasites of the mule deer in western Colorado in 1949, *S. parva* was found in 12 of the 31 caeca and large intestines examined. Thirty males were obtained from the 428 worms collected. The male of *S. parva,* the fourth known for the genus, is described in this paper.

Description of male of *S. parva* Dikmans, 1942

Body length 1.90–2.17 mm.; maximum width 1.01–1.27 mm.; width at level of esophageal bulb 0.070–0.116 mm. Diameter of head 0.041–0.047 mm. Total length of esophagus 0.243–0.278 mm.; width and length of esophageal bulb 0.055–0.078 mm. and 0.070–0.083 mm., respectively.

Mouth opening surrounded by 3 lips whose free margins are broadly oval and smooth (Fig. 2); tips of lips extend anteriorly beyond external wall of mouth (Fig. 6).

Tail typical of the genus, being rounded and blunt, with a round cuticular expansion. Four processes, one long lateral preanal pair and one short postanal pair, extend to the margin of the cuticular expansion (Fig. 4); each lateral process bears one terminal papilla, and each postanal process bears, in addition to the terminal papilla, a ventral subterminal one. Each of the 2 adanal processes bears one terminal and one subterminal papilla.

Dorsal terminal spike measures 0.008–0.012 mm. long (Fig. 5). Single spicule 0.060–0.074 mm. long; proximal end goblet-shaped. Gubernaculum 0.010–0.016 mm. long, clasps spicule snugly (Figs. 4, 5).

Host.—*Odocoileus hemionus hemionus* (Raf., 1817).

Location.—Caecum and large intestine.

Locality.—Rio Blanco, Gunnison, and Chaffee Counties, Colorado.

Specimens.—U. S. Nat. Mus. Helm. Coll. No. 46454 (Collateral-types), and Colorado A & M College Helm. Coll.

DISCUSSION

Mönnig (1932) divided the genus *Skrjabinema* Wereschtschagin, 1926 into the subgenera *Skrjabinema* and *Chilocrypta* on the basis of the position of the lips in relation to the anterior margin of the mouth wall. In the subgenus *Skrjabinema* the lips project anteriad from the external margin of the mouth wall (Fig. 6), whereas in *Chilocrypta* they are said not to project beyond it.

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The males of *S. ovis, S. oreamni,* and *S. parva,* the only known species of the subgenus *Skrjabinema,* are distinctly different from each other, and may be separated on the following morphological characters.

S. parva differs from the other 2 species in that it is the smallest, not exceeding 2.17 mm. in length, and in having the shortest spicule and gubernaculum (Table 1).

TABLE 1.—*Body measurements of the 4 known species of males of the genus* Skrjabinema

	Subgenus *Skrjabinema*			Subgenus *Chilocrypta*
	S. parva	*S. ovis*	*S. oreamni*	*S. rupicaprae*
	(mm.)	(mm.)	(mm.)	(mm.)
Body length	1.90 –2.17	2.46 –2.54	3.3 –3.7	1.54 –1.79
Width, maximum	0.101–0.127	0.128–0.186	0.175–0.210	0.108–0.112
Width at level of esophageal bulb	0.070–0.116	0.099–0.139	0.121–0.138	0.088–0.095
Head, width	0.041–0.047	0.034–0.044	0.047–0.057	0.040
Esophagus, total length	0.243–0.278	0.336–0.371	0.353–0.390	0.290–0.300
Bulb, diameter	0.055–0.078	0.060–0.086	0.097–0.109	0.048–0.054
Bulb, length	0.070–0.083	0.083–0.099	0.105–0.113	0.064–0.081
Terminal spine, length	0.008–0.012	0.009–0.017	0.020	0.025
Spicule length	0.060–0.074	0.073–0.086	0.077–0.085	0.080
Gubernaculum length	0.010–0.016	0.021–0.023	0.022–0.024	0.025

The spicule differs further in that the proximal end is goblet-shaped (Fig. 4), whereas in *S. ovis* and *S. oreamni* it is club-shaped.

The lips of each species differ in shape, as indicated by the unstippled areas in figures 1, 2, and 3. They are broadly accuminate in *S. ovis* and *S. oreamni.* The apex of each lip is distinctly notched in *S. ovis* (Fig. 3), whereas the tip of each lip in *S. oreamni* is continuous and without any indentation (Fig. 1). The free edge of the lips in *S. parva* is broadly oval in shape and without a marginal indentation (Fig. 2). In specimens freshly mounted in glycerine jelly, a labial pattern, probably formed by the pulp of the lips, could be seen in *S. oreamni* and *S. ovis* but not in *S. parva.* In *S. oreamni,* the pattern has a bilateral wing-shaped outline with a blunt median projection directed toward the apex of the lips and a median indentation on the side toward the base of the lips, as shown by the stippled area on each lip in figure 1. In *S. ovis,* the pattern is a line which forms in the central part of the lip an incomplete oval-shaped loop with the open portion facing the apex of the lips, as indicated by the dotted line in figure 3.

In his description and figure of the lips of the male of *S. oreamni,* Swales (1934) stated that they were T-shaped. Reexamination of specimens loaned by Dr. Swales from his original material revealed these T-shaped structures to be the labial pattern discussed above. In the specimens examined, the stem of the T-shaped structure described by Swales was not seen (Fig. 1). The lips of *S. oreamni* were represented by Swales (1934, Fig. 9) by the trifoliated figure shown in dotted lines, indicating a position inferior to the T-shaped structures and the anterior extremity of the mouth wall.

Further differences appear also in the number of adanal papillae in these 3 species. *S. parva* has 2 pairs, whereas *S. ovis,* according to Morgan (1930), Mönnig (1932), and our own observations, and *S. oreamni,* according to Swales (1934), have 3 pairs each.

S. rupicaprae belongs to the subgenus *Chilocrypta* Mönnig, 1932, and is the only male known from that group at the present. It differs from the males of the

3 species in the subgenus *Skrjabinema,* as listed above, in that the lips are enclosed by the mouth wall, which is a subgeneric character. Additional morphological features differentiating it from the males of the subgenus *Skrjabinema* are given in table 1 and in the key.

The present study showed the lips of the males of *S. ovis, S. oreamni,* and *S. parva* to be similar in structure, but markedly different from the lips of the females. Böhm and Gebauer (1930) made no statement regarding differences in the lips of the males and females of *S. rupicaprae.* This difference in the structure of the lips necessitates a revision of Mönnig's (1932) concept of the genus to include these characters.

Emendation of the genus *Skrjabinema* Wereschtschagin, 1926

Oxyuridae of small size. Cuticle inflated around anterior end. Body provided with lateral alae for greater part of its length. Two lateral and 4 submedian head papillae present. *Mouth surrounded by 3 lips. Each lip of the females is anchor-shaped* and bears on its inner surface a pair of tooth-like plates which project toward center of mouth opening. Intermediate lips present. *Lips of males not anchor-shaped, but broadly accuminate or oval, free margin smooth or with notch and without tooth-like plates.* Excretory pore behind level of esophagus. Vulva in anterior half of body. Tail of male with small lateral alae supported by 2 pairs of large, thickened ray-like processes, one pair preanal and one pair postanal, each ending in an acute point. Tail bears a number of small papillae. A single spicule and gubernaculum present. Parasites of ruminants.

The species of the genus *Skrjabinema* may be identified by means of the key which follows.

Key to the subgenera and species of the genus *Skrjabinema* Wereschtschagin, 1926

1. Females 2
   Males 7
2. Lips not enclosed by mouth capsule (subgenus *Skrjabinema* Mönnig, 1932) 3
   Lips enclosed by mouth capsule (subgenus *Chilocrypta* Mönnig, 1932) 5
3. Body 3.5–4.0 mm. long; tail 380–425 μ long; eggs 36–45 μ wide by 65–70 μ long. In deer (*Odocoileus hemionus*). ..... *S. parva* Dikmans, 1942.
   Body over 6 mm. long 4
4. Body 6.6–8.0 mm. long; tail 0.970–1.230 mm. long; eggs 27–34 μ wide by 43–63 μ long. In sheep (*Ovis aries*). ..... *S. ovis* (Skrjabin, 1915).
   Body 9.5–10.5 mm. long, tail 1.48–1.70 mm. long; eggs 30–35 μ wide by 53–57 μ long. In mountain goat (*Oreamnos americana*) and caribou (*Rangifer* sp.). ..... *S. oreamni* Swales, 1934.
5. Width of head 82 μ; length of esophagus 622 μ; length of tail 95–100 μ; excretory pore 824–915 μ and vulva 1.24–1.50 mm. from head end. In sheep (*Ovis aries*). ..... *S. alata** Mönnig, 1932.
   Width of head, length of esophagus, and length of tail distinctly less, and distance of excretory pore and vulva from head distinctly more than in *S. alata* 6
6. Excretory pore 1.28–1.37 mm. from head end; head 75 μ wide; esophageal bulb 146–150 μ wide. In steenbuck (*Rhapiceros campestris*). ..... *S. africana** Mönnig, 1932.
   Excretory pore 1.0–1.14 mm. from head end; head 60–68 μ wide; esophageal bulb 108–128 μ wide. In chamois (*Rupicapra rupicapra*). ..... *S. rupicaprae* Böhm and Gebauer, 1930.
7. Lips not enclosed by mouth capsule (subgenus *Skrjabinema* Mönnig, 1932) 8
   Lips enclosed by mouth capsule (subgenus *Chilocrypta* Mönnig, 1932) 10
8. Body 1.90–2.17 mm. long; free margin of lips broadly oval, without marginal notch (fig. 2), 2 papillae on each adanal process; spicule 60–74 μ long, proximal end goblet-shaped; gubernaculum 10–16 μ long. In deer (*Odocoileus hemionus*). ..... *S. parva* Dikmans, 1942.
   Body 2.46 mm., spicules 73 μ and gubernaculum 21 μ or greater in length; proximal end of spicule not goblet- but club-shaped; 3 pairs of papillae on adanal processes 9

* Males unknown.

9. Body 2.46–2.54 mm. long; free margin of lips with distinct notch at apex (fig. 3); head 34–44 μ wide; esophageal bulb 60–68 μ wide by 83–99 μ long; spicule 73–86 μ and gubernaculum 21–23 μ long. In sheep (*Ovis aries*). ..... *S. ovis* Skrjabin, 1915.
Body 3.3–3.7 mm. long; free margin of lips smooth (fig. 1); head 47–54 μ wide; esophageal bulb 97–100 μ wide by 105–113 μ long; spicule 77–85 μ and gubernaculum 22–24 μ long. In mountain goat (*Oreamnos americana*) and caribou (*Rangifer* sp.).
S. oreamni Swales, 1934.

10. Body 1.54–1.79 mm. long; head 40 μ wide; esophageal bulb 48–54 μ wide by 64–81 μ long; spicule 80 μ and gubernaculum 25 μ long. In chamois (*Rupicapra rupicapra*).
S. rupicaprae Böhm and Gebauer, 1930.

SUMMARY

1. Thirty males of *S. parva* Dikmans, 1942 were found in a collection of 428 worms obtained from 12 of 31 caeca and large intestines examined from mule deer, *Odocoileus hemionus hemionus* (Rafinesque, 1817), from western Colorado.

2. The male of *S. parva* is described and figured.

3. The lips of the male of *S. oreamni* Swales, 1934 are redescribed.

4. Inasmuch as the lips of the males of the subgenus *Skrjabinema* differ markedly from those of the females of the subgenera *Skrjabinema* and *Chilocrypta,* the generic concept is emended to include this difference.

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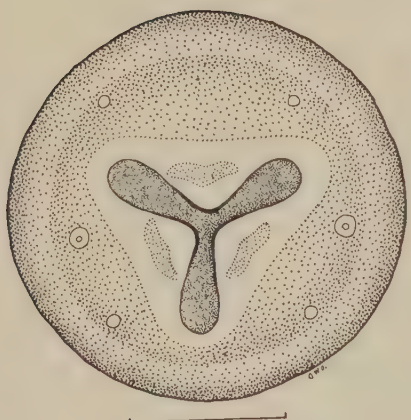

FIG. 1

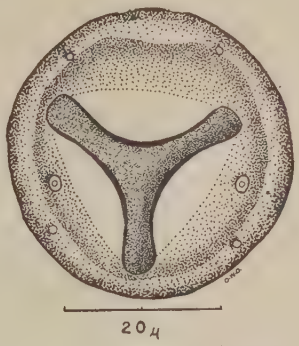

FIG. 2

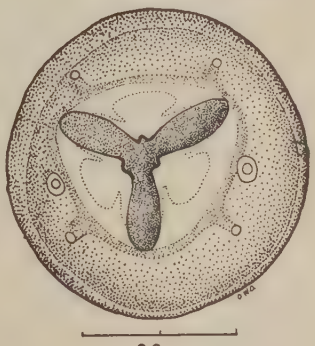

FIG. 3

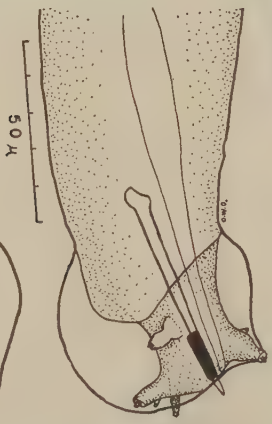

FIG. 5

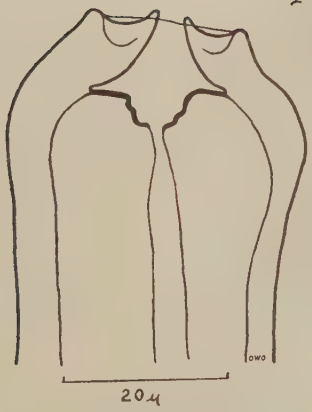

FIG 6

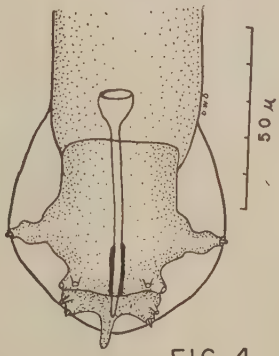

FIG. 4

EXPLANATION OF PLATE

FIG. 1. En face view of male of *S. oreamni* Swales, 1934.
FIG. 2. En face view of male of *S. parva* Dikmans, 1942.
FIG. 3. En face view of male of *S. ovis* Skrjabin, 1915.
FIG. 4. Ventral view of posterior end of male of *S. parva.*
FIG. 5. Dextral view of posterior end of male of *S. parva.*
FIG. 6. Median section through anterior end of male of *S. parva* showing shape of lips.

NOTES ON BOVINE SARCOSPORIDIOSIS[1]

Hsi Wang

American Meat Institute Foundation, The University of Chicago

One century after its discovery by Miescher (1843), *Sarcocystis* is known today as cosmopolitan, unicellular, muscle parasites which infect many species of animals representing practically every class of vertebrates (Blacklock and Southwell, 1931; Scott, 1930; Hegner, *et al,* 1938). Among the domestic animals the highest incidence is in sheep (Scott, 1943a, 1943b). A few cases were reported in cattle (Adams, 1935; Koffman, 1940; Scaglia, 1930; Thevenoz, 1932). These were mostly obtained from autopsy, and the parasites were found in the muscle of the larynx, tongue, oesophagus or myocardium. Recently, during an investigation of beef histology, by chance we found a typical case of sarcosporidiosis in the ribeye muscle *(Longissimus dorsi)* of two steers. The same parasite was encountered again in the *Triceps brachii* of another steer similarly studied. Since then, many similar cases of sarcocystic infection were uncovered in the *Longissimus dorsi* and *Semitendinosus,* two muscles which were investigated in our laboratories for other purposes. Up to now the parasite has been found in the *L. dorsi* in 36 carcasses and in the *Semitendinosus* in 5 carcasses out of a total of 48 beef carcasses of different grades examined.

The muscles infested with the sarcocysts were, in most cases, taken from carcasses in cold storage ranging from one to three days old when fixed. In the case of the first two steers, however, the *L. dorsi* muscle was fixed on a killing floor of the Chicago Union Stock Yards before the onset of rigor mortis. Thin pieces of this muscle were placed in two fixatives, Zenker-formol or Bouin's fluid, within 50 minutes after death of the animal. Sections, 8 micra in thickness, were made from celloidin-imbedded blocks and were stained with hematoxylin and eosin-azure II (Maximow). These sections show both the muscular tissue and the parasite in a fine histological state. The present account is based on materials found in the *L. dorsi* of these two cattle. There is no doubt that all the instances of infection concerned in this report are caused by the same species of *Sarcocystis* and that none of the cases involved an intensity high enough to be pathogenic to the host. The most severe cases had a density of about five cysts per half cubic centimeter of tissue.

RESULTS AND DISCUSSION

All the cysts were found within the limit of individual muscle fibers. The structure of the infected muscle fibers is not affected other than being distended as a result of growth pressure of the cysts (Figs. 2 and 3). Even the fibrils nearest the encysted parasites display details of normal cross striations (Fig. 4). The cysts ranged from small ovoid bodies (0.06 mm. by 0.04 mm.) to typical "Miescher's tube" (cf. Blacklock and Southwell, 1931; Hegner, *et al,* 1938)about 0.3 mm. in length (Figs. 1 and 3). The diameter of most cysts is approximately 0.03 mm. These cysts are not thick-walled; instead, only a thin membrane stained a light pink

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[1] A preliminary report was published in the *Anatomical Record* (Wang, 1949).

surrounds them (cf. Dubin and Wilcox, 1947). This membrane appears homogeneous, and not fibrillar, a lightly stained area immediately enveloping the lobules (loculi) of the deeply stained spores (Fig. 3). No septa (trabeculae) lead from it centrally to form the wall of the lobules (cf. Hewitt, 1933). Cysts reaching a dimension of 0.08 mm. in diameter (Fig. 2) became encapsulated. The capsule is roughly

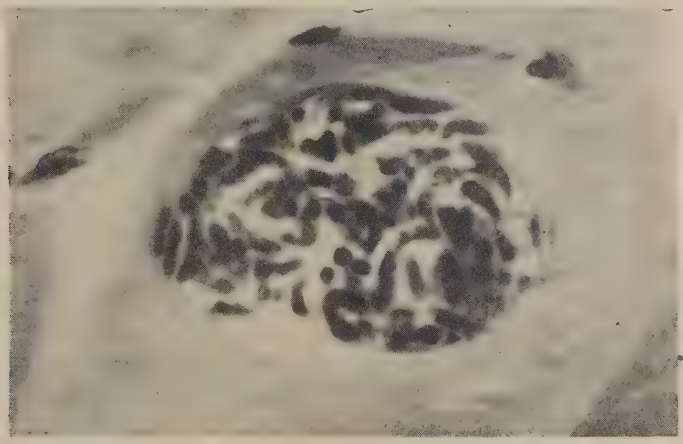

FIG. 1. A small sarcocyst with a thin membrane separating it from the substance of the muscle fiber in which it is lodged. A clear hyaline body and darkly stained nucleus in some of the spores are visible. × 1000.

one-tenth the diameter of the cyst in thickness, and reveals under high magnification distinct radial striations in its outer layer (Fig. 5), which comprises roughly four-fifths of the entire thickness (cf. Feng, 1932; Hewitt, 1933; Wiley, *et al,* 1904). Its inner layer, homogeneous throughout, seems to be similar to the membrane present in all younger cysts. Cyst walls intermediate between these two types were not found in our material.

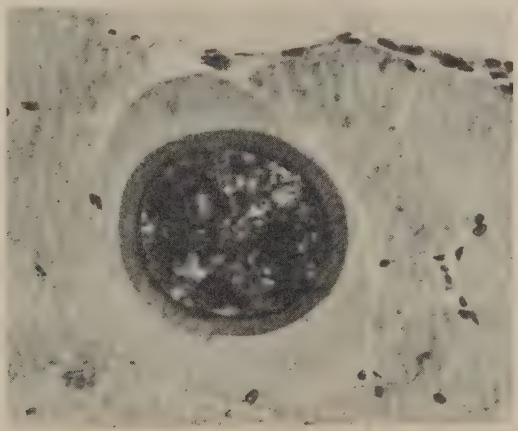

FIG. 2. X-section of a much older cyst with a thick striated capsule. Note the enlargement of the muscle fiber due to the parasite (cf. neighboring normal fibers). The spores are more densely packed than in younger cysts and they are contained in lobules. × 400.

FIG. 3. A typical "Miescher's tube" packed almost solid with spores. Along the edge of the cyst are scattered the sporoblasts, which are lightly stained and with a distinct central nucleus. ×300.

The origin of the capsule has long attracted the attention of both parasitologists and pathologists. Evidence at our disposal favors the host-origin. At very high magnification under oil immersion (2600 × original) a cyst that is about to be encapsulated reveals a complete, clear, peripheral ring which adjoins the host myofibrils. In longitudinal sections this ring further discloses definitive lines parallel to one another, and these lines appear to be in direct continuity with alternately the Z and M lines, the middle bands of, respectively, the isotropic and anisotropic disks of the myofibrils (Fig. 6). One is thus led to ask whether these lines might or might not represent the remnants of the myofibril disks to be finally transformed into the striations of the formed capsule. If one measures these striations (Fig. 5), he finds that each corresponds precisely with one complete cross striation of the myofibril (i.e., from one Z line to the next, which is approximately 2 micra). This interpretation of host origin of the capsule (cf. Blacklock and Southwell 1931; Scott, 1930), if proven correct, may advance our knowledge of the basic structure of the myofibril.

All cysts were heavily loaded with spores (sporozoites), both the number and density of which increased with age (Figs. 1 and 2). The size of spores, on the

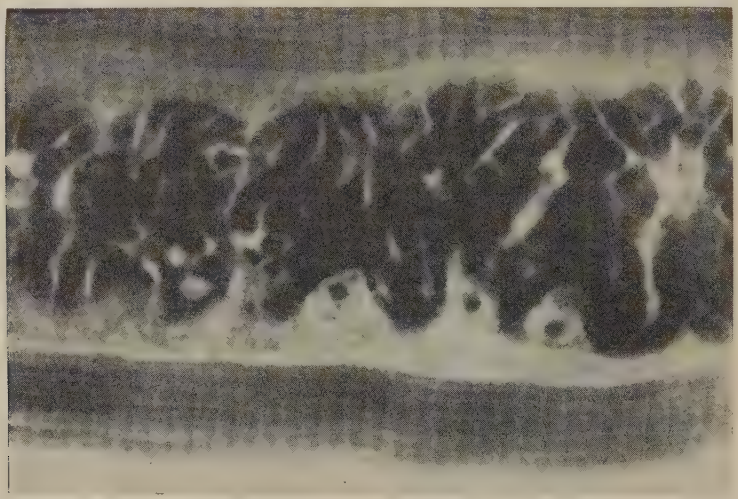

FIG. 4. A highly magnified portion (left end) of Fig. 3 to show four well demarkated sporoblasts, lobulated spores, and the contact relationship between the cyst and the infected muscle fiber. Note the absence of a capsule. ×1600.

FIG. 5. An enlarged portion of the cyst shown in Fig. 2 to show its striated capsule. The striations are approximately 2 microns apart, corresponding closely to the thickness of the myofibril disks (cf. Fig. 6). ×1735.

other hand, was relatively constant (9–11 micra in length) in all cysts except those of the capsulated ones, indicating that mature spores were produced even in small cysts and that the growth of a cyst involved a tremendous increase in the number of spores probably through multiplication of both the sporoblasts and spores (cf. Scott, 1930). The spores in the capsulated cysts are more numerous, but definitely smaller (7 micra or less) and less healthy looking. Their weak staining reaction portrays a beginning of disintegration (Fig. 2). Capsulation is, therefore, a sign of age brought on by the host as a means of eliminating the parasite (cf. Blacklock and Southwell, 1931; Edelmann, *et al*, 1943). This interpretation is in line with the origin of the striated layer of the cyst's capsule just discussed. However, neither calcification (Edelmann, *et al*, 1943) nor capsulated cysts with their interior par-

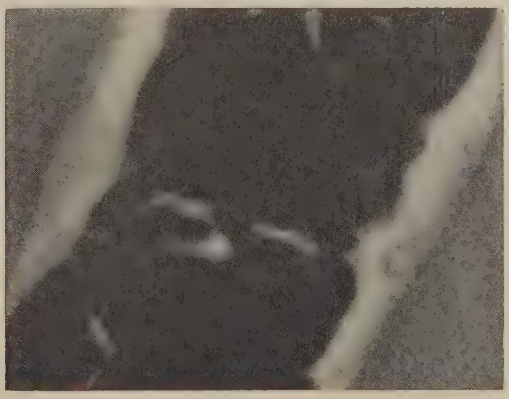

FIG. 6. An enlarged portion of a cyst intermediate in size between those shown in Figs. 4 and 5 to show a possible material relationship between the Z and M disks of the host myofibrils and the cyst's capsule under formation. ×1735.

(All sections from which these photomicrographs are taken are 8 micra thick, and are stained with hematoxylin and eosin-azure II. Those of Figs. 1 and 2 were fixed with Bouin's fluid; the rest, Zenker-formol.)

tially emptied through death of the centrally-located spores (Nowak, 1930; Scott, 1943a) was found in our material, so the fate of these aged cysts could not be determined.

Sporoblasts were absent from relatively young cysts and the capsulated ones (Figs. 1 and 2). They were present in fair numbers in the cysts of intermediate size (Figs. 3 and 4). These cells occupy a characteristic position in the cyst, always next to the cyst membrane, virtually resting on the latter (Fig. 4). They are somewhat spherical cells with a distinct central nucleus, seen either singly or sometimes in columns of two or three. This curious distribution of sporoblasts with respect to age of the cysts, if significant at all, suggests a continuous slowing down of the activity of the sporoblasts in the development of a cyst. The grouping of spores into definitive lobules (Figs. 2 and 4) occurs only in cysts of considerable size. The phenomenon has been linked to the development of septa from the inner layer of the capsule (Wenyon, 1926), but in the present case, its production may have simply resulted from the immense growth pressure developed under a rate of spore formation faster than could be accommodated by the host muscle fiber.

The structure of the spores (Fig. 1) bears resemblance to that described for *Sarcocystis tenella* (Nowak, 1930; Scott, 1943a; Wenyon, 1926), *S. muris* (Hegner, *et al,* 1938 and *S. hominis* (Blacklock and Southwell, 1931). Each spore is slightly bent into the shape of a banana with one end more pointed than the other. A clear oval hyaline body is present near the center of the cell while one end (usually the less pointed one) contains a mass of darkly stained granules among which the nucleus is situated (cf. Scott, 1943a).

Sarcosporidiosis, except in extreme cases (Scott, 1930 and 1943a), seldom produces any definitive symptoms of pathogenicity. This fact is best illustrated by the case of a monkey, which was normal in spite of a very severe degree of infection (Dubin and Wilcox, 1947). However, localized host reactions have been often observed (Dubin and Wilcox, 1947; Salomon, 1935). Many sections of the present material reveal degenerated muscle fibers or fibers undergoing atrophy. They were often accompanied by leukocytic (eosinophil) infiltration indicative of host inflammatory reaction. But inasmuch as no parasite was found directly connected with an inflammatory area, the phenomenon is believed to be in response to the degenerating muscle fiber rather than to the parasite itself (cf. Dubin and Wilcox, 1947). Concurring with Hertig (1934) and Gilmore, *et al* (1942), the writer did not observe any reaction around infected fibers.

Surprisingly enough, little or no morphological affinity was found between the present *Sarcocystis* and those which infected cattle (Skibsted, 1945), ox (Osterud and Bascom, 1928; Wenyon, 1926), or buffalo (Willey, *et al,* 1904). On the other hand, the present parasite presents a rather high degree of similarity to three cases of human sarcosporidiosis (Baraban and St. Remy, 1894; Feng, 1932; Naidu, 1928) on the basis of morphological details of the capsulated cysts (Feng, 1932, Fig. 3). Similarity between the present parasite and those infesting sheep (Blacklock and Southwell, 1931; Scott, 1943a; Wenyon, 1926), mouse (Hegner, *et al,* 1938), and pig (Kean and Grocott, 1945, Fig. 5) was also indicated. In line with these observations, one may recall the frequent demonstration of lack of host specificity in *Sarcocystis* infections. Thus, it has been shown by feeding experiments (Betegh and Dorcich, 1912; Darling, 1910a and 1910b; Erdmann, 1910; Negri, 1908a, b; Roder-

ick, 1929) that *S. tenella* can infect mouse, guinea-pig, hen, and duck. Similarly, identity of parasites was established in guinea-pig and man, between hog and cattle (Skibsted, 1945), and between guinea-pig and opossum. Finally, experimental transmission of the parasite from swine to mouse, dog, cat, chicken, and rat, and from these hosts back to swine was accomplished (Spindler, *et al*, 1946).

In view of these considerations, the writer is of the opinion that there is just one species of *Sarcocystis* (cf. Alexeiff, 1913; Wenyon, 1926) which possesses such a degree of adaptability as to be able to survive in many species of hosts, and that all existing differences observed in their morphology are caused by external factors too numerous to be discussed here (see Scott, 1930 and 1943a).

SUMMARY

Sarcocysts were found in the muscle fibers of *Longissimus dorsi* of two young steers, in the *Triceps brachii* of a third, and in *L. dorsi* and the *Semitendinosus* of more than two-thirds of 48 healthy beef carcasses of different grades. The young cysts were enveloped in a thin homogeneous membrane while the oldest ones possessed a striated capsule of considerable thickness. Microscopic details of host-parasite structural relationship supports host-origin of the capsule. The smaller cysts contained spores of fairly uniform size (10 micra) while those in the encapsulated cysts were smaller and slightly pyknotic in appearance. Sporoblasts were found only in the medium-sized cysts. Based on the morphology of cyst and spore, the present *Sarcocystis* reveals affinity to *S. tenella* (sheep), *S. muris* (mouse), and *S. homonis* (man), but not to those infesting ox and buffalo.

ACKNOWLEDGMENT

The writer wishes to express thanks to Dr. William Bloom for generously providing facilities in the early part of this investigation.

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A NEW SPECIES OF *PHYSALOPTERA* (NEMATODA: SPIRUROIDEA) FROM THE COTTON RAT*

STEWART C. SCHELL

The original representatives of this species were encountered in live-trapped cotton rats purchased from the Hegner Research Supply House in Sarasota, Florida. The parasites inhabit the stomach of the host. They feed in a compact group and cause the formation of a chronic ulcer. Approximately 20% of all cotton rats examined were found to be infected. The species was described from a large series of specimens of both sexes.

Physaloptera hispida n. sp.

Robust worms with fine cuticular striations; females pink with tiny flecks of brown pigment; males white and smaller than the females. Two semicircular lateral lips at anterior end, each provided with a lateral amphid; one externolateral tooth, three internolateral teeth; one subdorsal and one subventral papilla; papillae 12.2 μ in diameter; collarette absent.

Male: Length 30–42 mm.; width 0.9–1.4 mm. Length of muscular esophagus 0.56–0.73 mm.; of glandular esophagus 3.7–4.5 mm.; and of entire esophagus 4.2–5.3 mm. Deirids opposite to slightly asymmetrical, and 0.7–1.06 mm. from the anterior end; excretory pore 0.87–1.2 mm. from the anterior end; distance from lip to nerve ring 0.55–0.7 mm. Caudal end of male flexed, bluntly pointed, with caudal alae; four pairs of evenly spaced subventral pedunculate papillae, the two middle pairs the longest; three sessile preanal papillae and five pairs of sessile postanal papillae, the first two pairs on the posterior margin of the anus, the third pair asymmetrical (Fig. 7) with the left anterior to the right, the fourth pair asymmetrical with the left anterior to the right, the last pair opposite and close together. The spicules unequal in size, the right the longer, lanceolate and uniformly tapering toward the tip (Fig. 1) and measuring 390–549 μ; the left spicule blade-shaped (Fig. 2) and 341–477 μ in length.

Female: Length 53–64 mm.; width 1.9–2.0 mm.; uterus didelphys of the B type of Schulz (1927) or the 2-B type of Morgan (1943). Anterior muscular esophagus 0.64–0.69 mm., glandular esophagus 4.4–6.3 mm., length of entire esophagus 5.0–7.0 mm. Deirids as in the male, 0.67–0.91 mm. from the anterior end; excretory pore 0.84–1.08 mm. from the anterior end; lip to nerve ring distance 0.71–0.74 mm.; vulva 11–16 mm. from the anterior end. The phasmids lie halfway between the anus and tip of tail (Fig. 4). Eggs 24–30 μ by 40–52 μ, embryonated when laid.

Host: Sigmodon hispidus littoralis Chapman.
Locality: Vicinity of Sarasota, Florida.
Location in Host: Pyloric region of stomach.
Types, male and female, U. S. N. M. Helm. Coll. #46477.
Paratypes U. S. N. M. Helm. Coll. #46478.

DISCUSSION

Other species of *Physaloptera* associated with the cotton rat are *P. bispiculata* Vaz & Pereira, 1935 and *P. muris-brasiliensis* Diesing, 1861, redescribed by Ortlepp (1922). Morgan (1943) made *P. bispiculata* a synonym of *P. getula* Seurat, 1917, redescribed by Seurat (1937). The latter species was originally found in two species of rodents in North Africa but has never actually been taken from the cotton rat.

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* An extract from a thesis submitted to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the Degree of Doctor of Philosophy. The author is grateful for the assistance given by Dr. Lyell J. Thomas under whose direction the work was accomplished. The paper is a contribution from the Department of Zoology, University of Illinois, Urbana, Illinois. Present address of the author, the Department of Biological Sciences, University of Idaho, Moscow, Idaho.

The author was able to examine paratypes of *P. bispiculata* but types of *P. getula* were not available for study. A comparison of *P. bispiculata* and *P. hispida* revealed the following differences. In *P. bispiculata* the left spicule is longer than the right, and the third and fourth pairs of postanal papillae of the male are opposite. In *P. hispida* n. sp. the right spicule is consistently longer than the left, and the third and fourth pairs of postanal papillae are asymmetrical.

Measurements of species involved are tabulated below:

Species	Length ♀ mm.	Length ♂ mm.	Right Spicule	Left Spicule	Author and Date of Publication
P. bispiculata ...	27–55	25	400	460	Vaz & Pereira 1935
P. getula	18.5	16.3	350	480	Seurat 1917
P. getula	31	20.6	450	450	Seurat 1937
P. hispida	53–64	30–42	390–549	341–477	Schell 1950

All of the above species have a B type uterus of Schulz (1927) or the 2-B type of Morgan (1943). *P. muris-brasiliensis* may be distinguished from these species by the possession of an A type uterus of Schulz (1927) or 2-A type of Morgan (1943). The author encountered only *P. hispida* in Florida cotton rats.

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Explanation of Plate

All figures are reproductions of camera lucida drawings.

Fig. 1. Right spicule.

Fig. 2. Left spicule.

Fig. 3. Anterior end of adult male; ventral view, showing deirids, excretory pore and nerve ring.

Fig. 4. Phasmids on adult female.

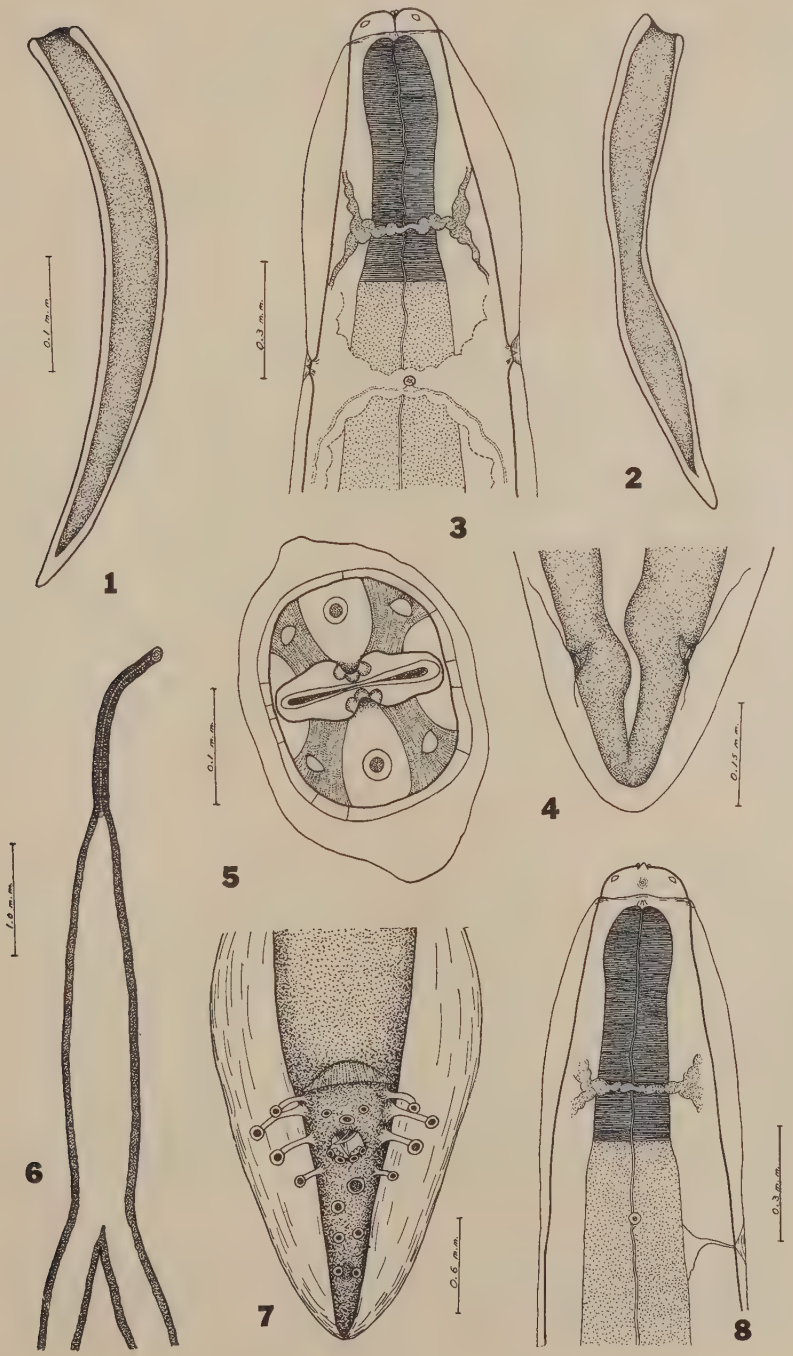
Fig. 5. *En face* view of adult female, showing externo-lateral and interno-lateral teeth, oral papillae, amphids and labial muscles.

Fig. 6. 2-B type uterus.

Fig. 7. Caudal end of adult male, ventral view, showing arrangement of postanal papillae.

Fig. 8. Anterior end of adult female, lateral view.

PLATE I



HELMINTHS IN RATS FROM PANAMA CITY AND SUBURBS

CARLOS CALERO M.,[1] PEDRO ORTIZ O., [2] AND LIONEL DE SOUZA[2]

Studies have been reported on the intestinal parasites of man and other animals (cats, dogs, equines, monkeys, opossums and capybaras) in Panama. No account is available for intestinal helminths of rats. The present communication records our findings on 400 rats captured in Panama City and suburbs. These rats were obtained through the Gorgas Board of Health Laboratory, Canal Zone.

If we consider the type of habitation of our poor sections of Panama City, the prevailing crowded conditions, and the enormous rat population infesting those sections, the need of this study is quite apparent.

MATERIAL AND METHODS

Four of the rats were *Mus rattus,* the others were *M. norvegicus;* 204 were males, 196 females. For the study, the stomach and the small and large intestines were removed. The three sections were opened separately and each section placed on a dish for macroscopic observations of the adult parasites which might be present. The final identification of these macroscopic parasites was always made with the help of the dissecting microscope. The stomach wall was carefully observed with the dissecting microscope to determine the incidence of infestation by *Gongylonema neoplasticum,* found in its wall. The contents of the appendix were examined for adult parasites, such as *Syphacia obvelata* and *Trichocephalus muris,* which normally are found in it. The walls and contents of the small intestine, leading from the pylorus, and of the large intestine, starting at the appendix, were studied microscopically with the dissecting microscope to determine the incidence of parasites. Finally, two smears were made, and the scrapings of the wall and the contents of the first duodenal section and of the cecum were studied microscopically to determine the incidence of ova, larvae and microscopic parasites. The liver was always examined for *Cysticercus fasciolaris* and flukes.

RESULTS

Specific diagnosis was made by the finding of both eggs and adult specimens in all cases except *Ascaris lumbricoides* where only eggs were found. Of 400 rats examined, helminth parasites were present in 333, or 83%. Of these, 100 were found to harbor only one species; 87 were hosts for two species; 85 for three species; 46 for four species; 14 for five species; and only 1 was found to harbor seven species. The highest incidence of infestation was by *Hymenolepis diminuta* 38%, followed by *Gongylonema neoplasticum* 28%, *Protospirura muricola* 29%, *Strongyloides ratti* 16%, *Moniliformis moniliformis* 16%, *Nippostrongylus muris* 13%, *Hepaticola hepatica* 12%, *Cysticercus fasciolaris* 10%, *Trichocephalus muris* 2%, *Syphacia obvelata* 1%, *Ascaris lumbricoides* 1%. Eggs of an unidentified trematode species were observed. *G. neoplasticum, H. hepaticola* and *P. muricola* are useful experimental species and a source of material as readily accessible as Panama City may be of interest to helminthologists.

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The authors are grateful to Col. Norman W. Elton, Director of the Gorgas Board of Health, Canal Zone, for furnishing the rats in the study here reported.

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GERMINAL DEVELOPMENT IN THE HETEROPHYID, *EURYHELMIS MONORCHIS* AMEEL, 1938*

D. J. Ameel, W. W. Cort, and Anne Van der Woude

INTRODUCTION

In the summer of 1948 we examined for larval trematodes 1282 specimens of *Pomatiopsis lapidaria* Say from Ann Arbor, Michigan, and found 18 infections of *Euryhelmis monorchis* Ameel, 1938. In two of these, there were only mother rediae, one with 6 and the other with 12. Most of the other infections were mature or old with free cercariae, but a few contained immature daughter rediae. We, therefore, had available for study of the germinal development of this species a number of different stages of both mother and daughter rediae.

The life cycle of *E. monorchis* was worked out by Ameel (1938). The cercariae of this species, like those of other heterophyids, escape from the daughter rediae before development is complete and finish their growth in the tissues of the snail. They penetrate frogs and tadpoles and become encysted in the skin and subcutaneous connective tissue. Adults were obtained from experimentally infected rats and a cat, and from naturally infected mink. Development in the definitive host is rapid. Specimens containing eggs were recovered from rats three and one-half days after experimental infection with metacercariae.

We were particularly pleased to obtain living material of the rediae of *E. monorchis* because it was the first heterophyid in which we have had the opportunity of studying the germinal development. Price (1940) has placed the genus *Euryhelmis* in the subfamily Apophallinae Ciurea, 1924 of the Family Heterophyidae Odhner, 1914. The adult of *H. monorchis* is rather unusual for this family because of the early degeneration of the male reproductive glands, and its cercaria differs from other known heterophyid cercariae in the absence of eye spots.

GERMINAL DEVELOPMENT

As already noted we found in one infection 12 immature mother rediae varying from less than 0.1 to about 0.5 mm. in length. All of these mother rediae were alike in having the body cavity crowded with embryos. The largest embryos were at the anterior end and they graded back to the smallest which were in contact with the germinal cells at the posterior tip of the body cavity. In the smallest mother rediae (Figs. 1–3) the germinal cells were rather numerous. They were in a compact group at the posterior end of the body cavity, and the anterior ones were contiguous with the smallest embryos. In the larger mother rediae (Figs. 4 and 5) the number of germinal cells had been considerably reduced, but there were none of them in which all the germinal cells had developed into embryos. In fact, all the mother rediae in this infection were immature, and no redial embryos had yet escaped from

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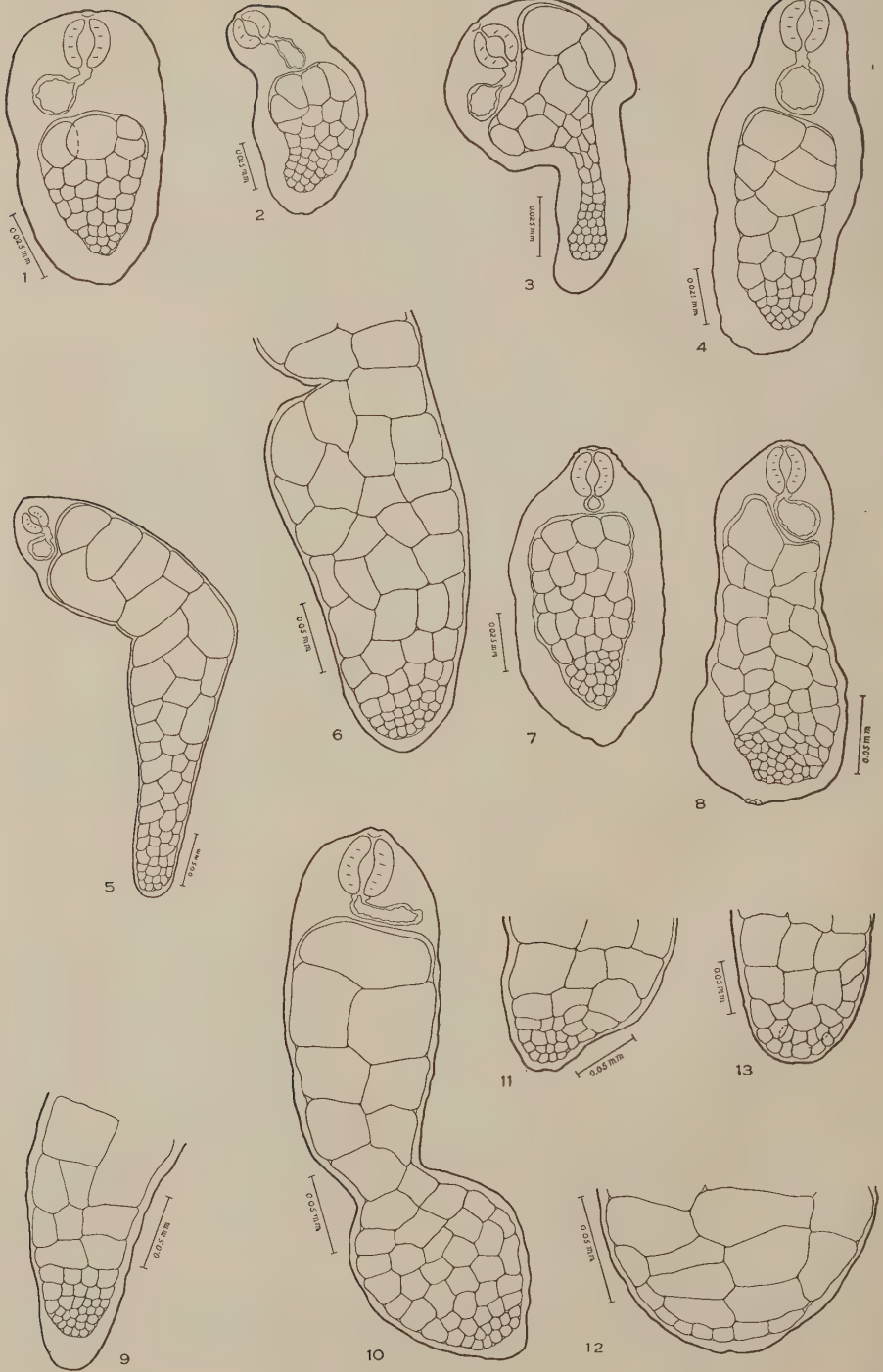
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any of them. The crowding of embryos in the body cavities of these rediae suggested that the development and growth of the embryos had proceeded synchronously with the expansion of the body cavity. In the largest mother rediae (Fig. 6) the daughter redial embryos at the anterior end of the body cavity were about 0.06 mm. in length. In them the pharynx and intestine were distinguishable and back of the posterior tip of the intestine in the primitive body cavity there was visible a morula-like group of germinal cells. This same type of germinal mass in redial embryos has been recently described for an echinostone species and for *Psilostomum ondatrae* (Cort, Ameel, and Van der Woude, 1949, figs. 4 and 8). It has also been found in redial embryos of *Halipegus eccentricus* (Ameel, Cort, and Van der Woude, 1949, fig. 10), and in those of *Paragonimus kellicotti* (unpublished observations).

The arrangement of the germinal material was also studied in immature daughter rediae. In them, as in the mothers, the body cavities were crowded with embryos with a considerable number of germinal cells at the posterior ends (Figs. 7–9). In a daughter redia 0.36 mm. in length which was evidently almost mature (Fig. 10) the number of germinal cells had been very considerably reduced. A few were still present in some mature daughter rediae (Fig. 11), but in most mature or old rediae only embryos remained (Figs. 12 and 13). Even when no germinal cells were present, the embryos usually filled the body cavity. The cercarial embryos at the anterior ends of the body cavities of these mature rediae escaped long before they were fully developed and completed their growth and development in the tissues of the snail.

While counts of the cercariae produced in single infections of *E. monorchis* are not available we obtained the general impression that the extent of the multiplication of individuals in its germinal sacs is rather restricted. In the two infections of mother rediae the numbers were 6 to 12 respectively. The numbers of daughter rediae in 8 mature infections, ranged from 44 to 186, with an average of 85. The comparatively small numbers of individuals produced in the rediae of this species is evidently due to a limitation in the period of multiplication of the germinal cells. No complex persistent germinal mass is present in which the cells continue to divide and produce new embryos throughout the life of the infection. In fact, the division of the germinal cells in *E. monorchis* appears to be limited to the early stages of the

Description of Figures

Drawings of rediae of *Euryhelmis monorchis* showing germinal development.

Fig. 1. Very immature mother redia, 0.096 × 0.050 mm.
Fig. 2. Very immature mother redia, 0.12 × 0.068 mm.
Fig. 3. Immature mother redia, 0.15 × 0.105 mm.
Fig. 4. Immature mother redia, 0.166 × 0.070 mm.
Fig. 5. Almost mature mother redia, 0.44 × 0.10 mm.
Fig. 6. Posterior half of almost mature mother redia, 0.50 × 0.095 mm.
Fig. 7. Very immature daughter redia, 0.13 × 0.07 mm.
Fig. 8. Immature daughter redia, 0.25 × 0.135 mm.
Fig. 9. Posterior end of immature daughter redia, 0.262 × 0.077 mm.
Fig. 10. Immature daughter redia, 0.36 × 0.10 mm.
Fig. 11. Posterior end of mature daughter redia, 0.56 × 0.13 mm; a few germinal cells remain.
Fig. 12. Posterior end of mature daughter redia, 0.45 × 0.13 mm; no germinal cells at posterior end of body cavity.
Fig. 13. Posterior end of mature daughter redia; no germinal cells remain.

development of the rediae, and possibly all the embryos produced are represented by unicellular components in the germinal mass at the time the first embryo starts to develop.

DISCUSSION

Cable (1934) made a cytological study of the germinal material in the rediae of *Cryptocotyle lingua,* a marine heterophyid for which the periwinkle, *Littorina littoria* serves as intermediate host. The youngest redia he observed was about 100 μ in length. In it, he considered as "primoridial germ-cells," cells lying among the strands of connective tissue within the region of the future body cavity. Their nuclei were 3–5 microns in diameter, and they could not be distinguished on the basis of size and staining reaction from the soma cells. Cable traced the development of these cells into typical germinal cells (l.c. pl. 32, figs. 1–4), with nuclei 7–8 microns in diameter, which were very similar to the mature oögonia of the adult worms. He also noted that the mature "germ-cells" increased in number by equal division and at later stages formed a distinct group at the posterior end of the body cavity of the rediae. These germinal cells had the diploid number of chromosomes which in this species is 12. The development of the germinal cells and the formation of cercarial embryos exhibited a distinct anterior-posterior gradient. In somewhat older rediae, the embryos toward the anterior end were always further along in development and between them and the germinal cells in the posterior end of the body cavity there was a continuous gradation of development. As the rediae became older the mature germinal cells were gradually used up in the production of embryos. Cable's figure 19 of plate 34 shows a section through the posterior end of a redia, in which a few germinal cells and a series of embryos are present.

It can be seen from the above discussion that the arrangement and development of the germinal material in the rediae of *C. lingua* is like that of *E. monorchis.* It may therefore, be suggested that this type of germinal development is characteristic of the HETEROPHYIDAE.

The type of germinal development found in *E. monorchis* and *C. lingua* appears to be adapted for the production of rather limited numbers of cercariae. This seems to be due to the limiting of the division of the germinal cells to the early stages of redial development. However, for *C. lingua* Rothschild (1939 and 1942) has reported the production of cercariae from the same infection over periods of several years. In her 1939 paper she stated that specimens of "*Littorina,* in the laboratory, have emitted cercariae for periods extending over three years, averaging about 300 cercariae per day." In her later note she reported that a very large specimen of *L. littorea,* 33 mm. in length, was still producing cercariae after seven years in the laboratory. At the end of the first five years this snail had given off approximately 5,500,000 cercariae with a mean daily average of 830. Later this figure rose to an average of 1,600 per 24 hours. It is difficult to explain such extraordinary fecundity, especially when we consider the simplicity of the mechanism of multiplication of germinal cells found by Cable in this species. Either the period of multiplication of germinal cells and the life of the rediae must be very greatly prolonged, or this species has become adapted for the production of repeated redial generations. It is interesting in this connection to note that only one generation of rediae has been found for *C. lingua.*

The method of multiplication of germinal cells of *E. monorchis* and *C. lingua* is very similar to that in the rediae of the two amphistomes that we have studied, *Allassostomum parvum* (Cort, Ameel, and Van der Woude, 1948) and *Megalodiscus temporatus* (Van der Woude, unpublished data) in which there also is no complex persistent germinal mass. In these forms, too, division of the germinal cells is limited to the early stages of redial development, and the embryos are tightly packed in the body cavity of the rediae in a series according to size. This same type of germinal development was also found in the daughter rediae of *Notocotylus urbanensis*[1] (Cort, Ameel, and Van der Woude, 1948) and in the mother and daughter rediae of *Nudacotyle novicia,* another representative of the NOTOCOTYLIDAE (Van der Woude, unpublished data.)

The type of germinal development in the rediae of the amphistomes and the notocotylids was considered as more primitive than that of the ECHINOSTOMATA in which there are complex persistent germinal masses which frequently are still producing new embryos in mature and even old rediae. The heterophyids also resemble the amphistomes and notocotylids in that the cercariae complete their development in the tissues of the snail host, are quite large, and in most cases have eyespots. It may perhaps be suggested that the heterophyids are rather primitive and are related to the amphistomes, perhaps being an offshoot from that group. The specialization of their large cercariae can be explained as an adaptation for penetration into a second intermediate host.

SUMMARY

Germinal development was studied in the rediae of *Euryhelmis monorchis* Ameel, 1938, a representative of the HETEROPHYIDAE. In immature mother and daughter rediae of this species the body cavity is packed with embryos in graded series with the germinal cells at its posterior tip. In most of the mature and old daughter rediae examined, all the germinal cells had developed into embryos. No complex germinal masses are present as in the rediae of the ECHINOSTOMATA and certain other trematode groups which can produce new embryos throughout the life of the infection. This type of germinal development in *E. monorchis,* which is like that described by Cable for another heterophyid, *C. lingua,* indicates that the multiplication of germinal cells is limited to the early development of the rediae. This is also characteristic of the amphistomes and notocotylids. This similarity in germinal development, and the fact that the cercariae require a period of growth outside the rediae in the tissues of the snail to complete their development, suggests the possibility that the HETEROPHYIDAE may be a rather primitive group related to the amphistomes.

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[1] It should be noted that we have come to the conclusion that we were probably mistaken in describing and drawing a complex germinal mass in the mother redia of *N. urbanensis* (l.c. fig. 6). The position of this mass is different from that of other rediae, and it seems surprising that the germinal development of the mother rediae in this species should differ from that of the daughters. We had a chance to study only two rather old mother rediae of this species, and may have mistaken a disintegrating embryo for a germinal mass.

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STUDIES ON SOME NORTH AMERICAN SHREW CESTODES

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Department of Biological Sciences, Purdue University, Lafayette, Indiana.

In spite of the importance of the various species of shrews to the biotic community, several aspects of their ecology remain very incompletely known. The helminths parasitic in the more conspicuous small mammals in North America have been studied more or less completely, but those occurring in shrews have been largely disregarded. This situation is particularly inappropriate when one considers that shrews often constitute the most abundant mammalian group in a given area. We have attempted to secure an adequate amount of shrew helminth material for study, and it is the purpose of this paper to present observations resulting from this work.

There have been, apparently, three species of cestodes recorded so far from North American shrews; these are *Hymenolepis anthocephalus* Van Gundy, 1935, *Diorchis reynoldsi* Jones, 1944, and *Protogynella blarinae* Jones, 1943. These parasites have been reported from the short-tailed shrew, *Blarina brevicauda* Say sspp. It seems that the parasites of the smaller shrews have been entirely neglected. This in part may be due to the difficulty with which good shrew material is obtained, since the animals undergo extremely rapid decomposition after death, often making cestodes useless for study. Taxonomic difficulties with the host animals themselves also may be considerable, particularly where several species occur in the same region.

For several years the senior author has collected helminths from shrews, but in some cases good material has been obtained only after repeated attempts. Most of the shrew material with which we are concerned was collected in the Central States. During the summer of 1948 the writers collected helminths from a number of shrews in the Jackson Hole region of Wyoming. Additional material has been obtained from the western provinces of Canada and from Alaska. Material was secured from the following species of shrews: *Blarina brevicauda, Sorex cinereus, S. vagrans, S. obscurus, S. articus,* and *Microsorex hoyi.* Subspecific names of the hosts are not considered here, except where mentioned in connection with a specific cestode. Most of the taxonomic work regarding the shrews, particularly the Alaskan forms, has been done by the senior author at the U. S. National Museum.

The cestodes which we have so far observed in shrews are considered separately. Of these, 4 species are described as new.

Protogynella blarinae Jones, 1943

(Figs. 1 and 2)

The genus *Protogynella* was erected by Jones (1943) for a very small cestode taken from *Blarina brevicauda* in Virginia. We collected this species from the same host in southern Wisconsin, although it was not found commonly there. While we are not able to either corroborate or refute certain of Jones' statements, it is evident that certain details were overlooked by him. *Protogynella blarinae* was described as having a "sac-like, unarmed" rostellum. Careful study of this cestode has revealed that the rostellum is actually armed with minute hooks. The number of hooks appears to be 46, and their length is about 4.0 μ (Figs. 1 and 2). This added knowledge regarding *P. blarinae* requires modification of the generic concept. Jones' failure to include

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a drawing of an entire mature segment causes some difficulty in understanding the morphology of this species; it is possible that certain other characters have been incorrectly interpreted, but conclusive evidence must be derived from the study of material in unusually favorable condition.

Diorchis reynoldsi Jones, 1944

The cestode was recorded by Jones (1944) from *Blarina brevicauda,* taken in Virginia. We have never observed this cestode in *Blarina,* nor in any other shrew species. It appears to have a restricted distribution.

Hymenolepis anthocephalus Van Gundy, 1935

Hymenolepis anthocephalus was commonly observed in *Blarina brevicauda* in the states of Ohio, Michigan, and Wisconsin. It is the largest cestode so far found in North American shrews. Apparently this species is restricted in occurrence to *Blarina,* since we have never taken it from shrews of other genera, even though they were collected from the immediate locality where infected specimens of *Blarina* were common.

Hymenolepis blarinae n. sp.

(Figs. 3 and 4)

Diagnosis: Strobila usually about 90 mm. long; greatest width, slightly over 1.0 mm. attained in gravid segments. Margins of strobila serrate; segments, 350 to 430 in number, usually wider than long, with fully gravid segments nearly square. Scolex about 250 μ in diameter; well developed and strongly set off from neck. Suckers about 125 by 190 μ. Rostellum not prominent; armed with 10 hooks, 33 μ in length. Ventral excretory canals up to 56 μ in diameter; dorsal canals, situated lateral to former, 5 μ in diameter. Genital pores unilateral, dextral; situated near the middle of segment. Cirrus sac from 110 to 130 μ long by about 16 μ wide. Cirrus spinose. Testes in mature segments about 50 μ in diameter; single anterior testis more or less between two posterior testes. Ovary elongate, with long axis transverse. Vagina posterior or ventral to cirrus sac. Vitelline gland posterior to ovary, near posterior margin of segment. Mature segments not observed posterior to first ¼ of length of strobila. Uterus first appears as transverse body in anterior part of segment; bilateral enlargements are formed, which enlarge until most of segment between excretory canals is filled by ovoid gravid uterus. Eggs apparently spherical, about 40 μ in diameter; distortion resulting from fixation has prevented accurate measurement. Larval hooks 19 μ long.

Host: Blarina b. brevicauda (Say).

Locality: Madison, Wisconsin.

Habitat: Small intestine.

Type: A slide bearing a complete specimen has been deposited in the Helminthological Collection of the U. S. National Museum, slide No. 47316.

Of other shrew cestodes belonging to the genus *Hymenolepis,* only one, *H. scutigera* (*Dujardin,* 1845), has 10 hooks which overlap in size those of the present species. These species, however, are readily differentiated on the basis of hook shape, in addition to other morphological characters.

Hymenolepis parva n. sp.

(Figs. 5 and 6)

Diagnosis: Strobila from 3 to 5 mm in length; greatest width, attained in gravid segments, about 300 μ. Margins of strobila not serrate. Segments from 125 to 150 in number. Scolex from 160 to 190 μ in diameter, markedly set off from neck. Suckers about 130 μ in diameter. Rostellum well developed; armed with 10 hooks 34 to 40 μ long. Ventral excretory canals about 8 μ in diameter. Genital pores unilateral, dextral; situated near middle of segment. Cirrus sac from 60 to 96 μ long by 12 to 22 μ wide. Cirrus spinose; about 25 μ long when protruded. External seminal vesicle present; in majority of cases it turns back ventrally upon cirrus sac, but may continue straight; in latter case gives appearance of cirrus sac extending across 2/3 of segment. Testes 25 to 35 μ in diameter in mature segments; situated in straight line. Ovary somewhat elongate, transverse, situated near middle of segment. Vitelline gland posterior and aporad to ovary, near posterior margin of segment. Vagina posterior to cirrus sac; seminal receptacle not prominent. Uterus first appears as transverse body which gradually enlarges to fill entire gravid segment. Eggs spherical, from 20 to 25 μ in diameter.

Host: Sorex c. cinereus Kerr. Also recorded from *S. cinereus streatori* Merriam (Anchorage, Alaska); *S. vagrans monticola* Merriam (Jackson Hole, Wyoming); *S. o. obscurus* Merriam (Tolugak Lake, arctic Alaska — lat. 68° 24′ N., long. 151° 26′ W.); *S. obscurus alascensis* Merriam (Juneau, Alaska).

Locality: Madison, Wisconsin.

Habitat: Small intestine.

Type: A slide bearing whole-mounts of paratype material has been deposited in the Helminthological Collection of the U. S. National Museum, slide No. 47317.

Hymenolepis parva appears to resemble most closely *H. scutigera* (Dujardin, 1845), the only other species of soricid *Hymenolepis* having 10 hooks of overlapping size. These two cestodes also have similar hook shapes. These species, however, may be differentiated on the basis of egg size and other morphological characters. Baylis (1934) found *H. toxometra* Baer, 1932, to be identical with *H. scutigera*. According to Baylis, uterus shape (an arc) and the greatly elongated gravid segments to differentiate *H. scutigera* from all other shrew species; this also applies to *H. parva*.

Hymenolepis schilleri n. sp.

(Figs. 7 and 8)

Diagnosis: Strobila length from 20 to 25 mm.; greatest width attained in terminal gravid segments, about 1.5 mm. Margins of strobila slightly serrate. Segments, about 300 in number, very much wider than long through strobila. Scolex strongly developed, from 410 to 420 μ in diameter. Suckers about 70 by 80 μ. Rostellum powerful; armed with 22 hooks 27 to 30 μ in length. Excretory canals markedly undulating; dorsal canal, about 8 μ in diameter, situated dorsal to proximal margin of ventral canal; latter 16 to 40 μ in diameter. Genital pores unilateral, dextral; situated in anterior third of margin of segment. Cirrus sac from 110 to 140 μ long by about 16 μ wide. Cirrus aspinose. Testes arranged in triangle near center of segment; two testes on same level, with third posterior to and between them. Ovary elongate, transverse; situated near anterior margin of segment. Vagina posterior to cirrus sac; large seminal receptacle may extend nearly to midline of segment. Vitelline gland transversely elongate; situated posterior to and parallel with ovary, near center of segment. Uterus first seen as transverse, rosette-like sac which gradually enlarges to fill entire gravid segment. Eggs from 21 to 24 μ in diameter.

Host: *Sorex c. cinereus* Kerr.

Locality: Madison, Wisconsin.

Habitat: Small intestine.

Type: A slide bearing paratype material has been deposited in the Helminthological collection of the U. S. National Museum, slide No. 47318.

Of other soricid cestodes of the genus *Hymenolepis*, *H. spinulosa* Cholodkowsky, 1912, *H. magnarostellata* Baer, 1931, *H. pistillum* (Dujardin, 1845), *H. maclaudi* Joyeux and Baer, 1928, *H. macroscelidarum* Baer, 1926, *H. furcata* (Stieda, 1862), and *H. uncinata* (Stieda, 1862) all have a hook number approximating that of *H. schilleri*. However, according to published descriptions, only *H. magnarostellata*, *H. maclaudi*, *H. pistillum*, and *H. furcata* actually overlap *H. schilleri* in hook number. Of these, only *H. magnarostellata* and *H. furcata* overlap in hook length. The latter two species as well as the others mentioned above are readily differentiated from the present species on the basis of hook shape, in addition to other morphological characters.

This cestode is named in honor of Mr. E. L. Schiller, who has been of aid in securing supporting material for this study.

Hymenolepis falculata n. sp.

(Figs. 9 and 10)

Diagnosis: Strobila from 30 to 40 mm. long; greatest width, attained in gravid segments, about 700 μ. Margins of strobila slightly serrate; segments, from 220 to 270 in number, slightly wider than long to nearly square. Scolex relatively small; from 180 to 220 μ in diameter; suckers about 50 by 75 μ. Rostellum well developed; armed with 12 hooks from 22 to 25 μ in length. Ventral excretory canals 32 to 64 μ in diameter; dorsal canals, situated directly dorsal to latter, about 8 μ in diameter. Genital pores unilateral, dextral; situated in anterior half of margin of segment. Cirrus sac elongate from 130 to 150 μ long by 16 to 24 μ in diameter. Cirrus spinose; about 70 μ long when protruded. Testes arranged in triangle; two situated antero-poral and one postero-aporal. Testes about 80 μ in diameter in mature segments. Ovary somewhat ovoid, with incised posterior margin; situated near middle of segment. Vagina posterior to cirrus sac; seminal receptacle not prominent. Ovoid vitelline gland situated in notch at posterior margin of ovary. Early uterus appears as wreath-like band completely surrounding female reproductive organs; open center of uterus persists until well toward end of strobila, when it disappears and the uterus fills entire gravid segment. Eggs ovoid; about 36 μ in length. Accurate measurement of eggs prevented by distortion resulting from fixation.

Host: *Sorex c. cinereus* Kerr.
Locality: Madison, Wisconsin.
Habitat: Small intestine.
Type: A slide bearing paratype material has been deposited in the Helminthological collection, U. S. National Museum, slide No. 47319.

Only two species of soricid cestodes of the genus *Hymenolepis* having 12 hooks have been described; these are *H. scalaris* (Dujardin, 1845) and *H. dodecantha* Baer, 1925. The hooks of both of these exceed those of *H. falculata* in length, and both differ in hook shape. The wreath-like shape of the early uterus of the latter serves to distinguish it readily from related species.

DISCUSSION

In addition to the 4 species described in the present paper, there are apparently 25 species of *Hymenolepis* which parasitize various shrews. Of these, only one, *H. anthocephalus* Van Gundy, 1935, has been described or recorded from North American hosts. It would seem, from present knowledge, that cestodes in North American shrews are strictly North American species, with none of the Eurasian forms represented insofar as the genus *Hymenolepis* is concerned. This is in contrast to certain other mammalian groups (*e.g.* microtine rodents) where Eurasian species occur more or less commonly, particularly in arctic-alpine regions. One of us (R. R.) has at present a large number of preserved shrews from the coast of the Bering Sea and Nunivak Island, the examination of which may divulge further information in this connection. Further studies on shrew helminths will be presented at a later date.

A key to the cestodes recorded from North American shrews has been prepared, based mainly on hook characters.

Key To Species Of Cestodes In North American Shrews

1. Scolex armed 3
   Scolex unarmed 2
2. Scolex relatively large and globular; no rostellum .... *H. anthocephalus* Van Gundy, 1935
3. Rostellar hooks more than 40 4
   Rostellar hooks less than 40 5
4. Strobila length about 1 mm; about 46 minute hooks present
   Protogynella blarinae Jones, 1944
   Strobila length 7 to 10 mm; about 100 minute hooks ..... *Diorchis reynoldsi* Jones, 1943
5. Rostellar hooks 22 in number; segments much wider than long ..... *H. schilleri* n. sp.
   Rostellar hooks 12 in number; pre-gravid uterus wreath-like in appearance
   H. falculata n. sp.
   Rostellar hooks 10 in number 6
6. Strobila from 7 to 10 mm. long; hook length 34 to 40 μ long ..... *H. parva* n. sp.
   Strobila about 90 μ long; hook length 33 μ ..... *H. blarinae* n. sp.

SUMMARY

The cestodes of North American shrews are discussed, and 4 species, *Hymenolepis blarinae, H. parva, H. schilleri,* and *H. falculata,* are described as new. Rostellar hooks are reported for the first time from *Protogynella blarinae* Jones, 1944; this requires emendation of the generic diagnosis. Various host- and distribution records are also reported.

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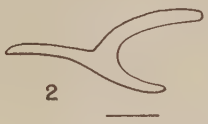
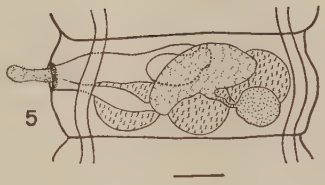
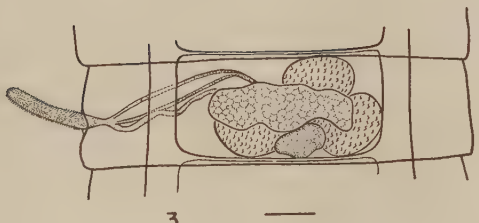
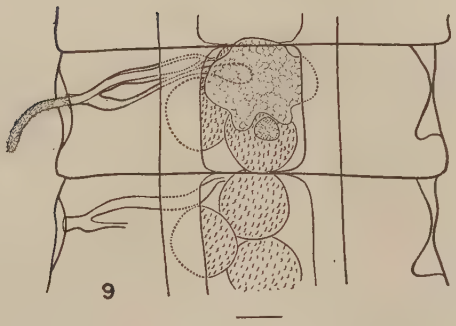
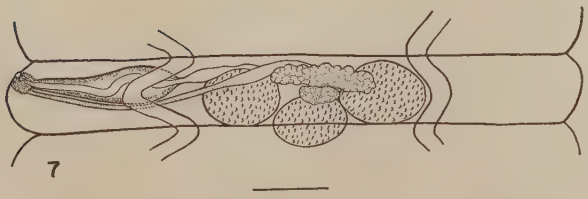
PLATE I











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Explanation Of Plate

Fig. 1. Scolex of *Protogynella blarinae* showing hooks on retracted rostellum. Scale has a value of 100 μ.

Fig. 2. Rostellar hook of *P. blarinae*. Scale has a value of 1 μ.

Fig. 3. Mature segment of *Hymenolepis blarinae* n. sp. Scale has a value of 40 μ.

Fig. 4 Rostellar hook of *H. blarinae*. Scale has a value of 10 μ.

Fig. 5. Mature segment of *H. parva* n. sp. Scale has a value of 20 μ.

Fig. 6. Rostellar hook of *H. parva*. Scale has a value of 9 μ.

Fig. 7. Mature segment of *H. schilleri* n. sp. Scale has a value of 60 μ.

Fig. 8. Rostellar hook of *H. schilleri*. Scale has a value of 10 μ.

Fig. 9. Mature segment of *H. falculata* n. sp. Scale has a value of 80 μ.

Fig. 10. Rostellar hook of *H. falculata*. Scale has a value of 10 μ.

ESTABLISHMENT AND PATHOLOGY OF GORGODERID INFECTIONS IN ANURAN KIDNEYS

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INTRODUCTION

Adult digenetic trematodes of the subfamily GORGODERINAE are commonly called bladder flukes because of their seeming preference for residence in the urinary bladders of cold-blooded vertebrates. That this popular name may be a misnomer and that these flukes are not limited to habitation in urinary bladders has been known since Sinitsîn (1901, 1905), Nybelin (1926), and Lutz (1926) found them in the ureters of fishes and amphibians. Other workers, Joyeux and Baer (1934), Odlaug (1937), Rankin (1939), and Goodchild (1945, 1948), have also recovered them from non-vesicular sites and, in part, have commented on the significances of these deviations from the usual infection pattern.

Analyses of establishment and pathology of infection in anuran mesonephroi and ducts, and variation in adults attaining full maturity in the kidney have so far not been available in the literature. It is, therefore, the purpose of the present paper to supply information on these phases of the problem.

MATERIALS AND METHODS

Juveniles and sexually-mature adult flukes have been taken from the Wolffian ducts and mesonephroi of frogs captured in the town of Falmouth, Massachusetts (*Gorgoderina attenuata* and *Gorgodera amplicava* from *Rana catesbeiana, et al.*); Greene County, Missouri (*Gorgodera amplicava* from *R. catesbeiana*); and Dade County, Florida (*Gorgodera amplicava* from *R. grylio*), in the years since 1943.[1]

Some of these worms have been prepared as whole mounts after fixation with Gilson's fluid and staining with paracarmine and precipitated borax-carmine. Sections have also been prepared of whole kidneys and ureters to show immature and mature worms *in situ*. Routine histological techniques were used to imbed the parasitized organs in paraffin. Frontal and cross sections were stained with standard alum haematoxylin and Heidenhain's iron-alum haematoxylin with and without erythrosin as a counterstain.

The author is indebted to Dr. Louis Olivier, National Institutes of Health, Bethesda, Maryland, who kindly loaned five serially sectioned and stained tadpoles of *Rana pipiens*. These tadpoles were naturally infected (Douglas Lake region of Michigan) with young gorgoderid trematodes.

INTERPRETED OBSERVATIONS

The establishment of gorgoderid infections in anurans has been traced experimentally by Rankin (1939) and Goodchild (1945, 1948). Briefly the usual post-

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[1] Dr. Elon E. Byrd, The University of Geeorgia, Athens, Georgia, in personal communication, has stated that bullfrog kidneys which he has examined and sectioned have also revealed developmental stages of gorgoderid worms in the kidney substance.

metacercarial sequence is, 1) excystment in the small intestine, 2) posterior migration along the mucosa of the colon and cloaca, 3) invasion of urogenital ducts which open into the cloaca with preference shown, in order, for the Wolffian ducts, the cloacal bladder, and, in the case of female hosts, the oviducts. Slow uncomplicated growth to sexual maturity ensues in juveniles reaching the bladder; the fate of worms moving into the oviducts is not known.

Juvenile flukes are found in greatest numbers in the Wolffian ducts, the upper ends of which are often swollen by tightly packed masses of worms. Earlier the writer (1948) reported that young worms were limited to the excretory ducts; this statement is erroneous and Odlaug's (1937) discovery of immature *Gorgodera amplicava* in mesonephric tissue is corroborated. Apparently most worms, even those temporarily residing in the kidney tissue are able to extricate and to establish themselves in the urinary bladder where they attain sexual maturity. Others, however, become imprisoned by encapsulation in the kidney and remain trapped there during their adult life.

Invasion, by juveniles, of interstitial mesonephric tissue or mesonephric tubules occurs from the ureter. In either case prompt host reaction induces a slight hyperemia and the initiation of encirclement by connective tissue cells. The most conspicuous early connective tissue elements involved are fibroblasts and diapedetic granulocytes, apparently identical to those occurring in the lumina of mesonephric blood vessels (Fig. 6). The granulocytes move into contact with the cuticula of the young worm and arrange themselves into a single layered capsule (Fig. 7). Continued infiltration, differentiation of the initial granulocytes, and cellular multiplication produce ultimately a multilayered capsular wall (Figs. 9 and 10). This cellular augmentation is accompanied by rather constant histological changes of the wall. At first, the wall adjacent to the cuticula of the worm is squamous-like and composed of granulocytes and elongated fibroblasts (Fig. 6). Next follows the formation of an internal cuboidal epithelium apparently by differentiation and redistribution of the granulocytes (Figs. 7 and 8). This layer is capable of secreting a granular mucoid-like substance always to be found in the capsular cavity and in the caeca of the parasite (Figs. 4, 6, 8, and 10). Outside the cuboidal layer (Fig. 8) is a persistent but thicker fibroblastic layer with conspicuous matrical fibers. In life these fibers impart a whitish color and toughness which aids location of encapsulated worms by sight or feel in case teasing is employed. Rarely two worms may be encapsulated together.

The persistently secretory internal epithelium in the fully-formed capsular wall is a "transitional" (in the sense that term is used for the mucous membrane of the larger urinary passages) epithelium (Fig. 10), evolved from the preceding cuboidal one by cell proliferation and centripetal growth. Invariably the layers nearest the parasite consist of secretory columnar cells, the free ends of which are packed with granules; the middle zone is composed of irregular polyhedral cells; basally the proliferous layer of rather regular cuboidal cells persists. The perifibrous zone remains unthickened but possesses more strongly developed fibers. Histologically the whole capsular wall is almost the equivalent of that of the Wolffian duct after irritative hyperplasia has thickened the latter in heavily infected specimens.

It is not known how long a time is required for encapsulated worms to reach

sexual maturity. Eventually, however, normal, fully-embryonated eggs containing miracidia are produced and are discharged into the capsular cavity. These ripe eggs are invariably seen in the cavities of sectioned capsules and in the mesonephric tubules (Fig. 4). Furthermore, similar ripe eggs have been identified in sections of the ureter, and these eventually could reach the outside to infect the next host. Encapsulated adults are therefore reproductively effective. Some eggs, however, are trapped in interstitial tissue and undergo gradual degeneration.

Massive infection in the kidneys and ureters is apparently harmful to the host. Frogs parasitized with several hundred juvenile worms are sluggish and obviously sickly. With heavier infection, death may result. In such cases the kidneys are hyperemic and purplish and undoubtedly traumatized because of the heavy influx of parasites. Death may be due to injurious histolytic wastes and uremia caused by kidney failure. Gorgoderid parasites may thus have economic importance in regions exploiting a native frog population or in commercial "frog farms."

Although young bladder flukes, *Gorgodera amplicava* and *Gorgoderina attenuata,* have been encountered in the mesonephroi and ducts, only the latter species apparently becomes sexually mature there. Excapsulated adults (Figs. 2 and 3) are markedly different in behavior and in appearance from those found in the urinary bladder (Fig. 1) in that they are weaker, flabbier, less mobile, smaller, and more attenuated. When they are lifted on needles they hang flaccid and non-resisting; in hanging they appear incapable of supporting their own weight, the body slowly elongating. They are transparent in contrast to bladder forms which are opaque and they tend to retain their transparency even after fixation and before clearing. The ventral sucker is smaller than normal, and the testes may be small enough to be inconspicuous (Fig. 2). Miracidia from adults residing in the kidney are identical in size and structure to those produced by bladder inhabiting specimens. Uterine coils, having the same postacetabular course in the two types of adults, are less distended with eggs and are more easily traced in adults from the kidney (Figs. 2 and 3).

The figures of adults have been drawn roughly to the same length for purposes of quick comparison of the various organs and organ systems. Organ sizes may readily be obtained by measurements from the figures and, therefore, will not be given here.

Even though the encapsulated worm is secure, with no possibility of being eliminated from the host such as reported for bladder forms by Goodchild (1948), the organism does cling to the inner wall of the capsule by the muscularly active oral and ventral suckers (Fig. 4). Peculiar spherical, brownish hyaline concretions with radiating lines, and of unknown origin and function, have been found in the acetabular cavities of several imprisoned parasites.

Adult *Gorgoderina attenuata* attaining sexual maturity in the cloacal ends of the Wolffian ducts are intermediate in opacity and mobility between encapsulated and bladder forms and uniquely are greatly elongated, thin and circular in cross section. As many as four sexually mature adults have been recovered from a kidney and eight from a ureter of *Rana catesbeiana.*

An unusual and as yet unexplained deviation from the known patterns of life

cycles in anuran gorgoderid parasites has been found in slides kindly loaned to the writer by Dr. Louis Olivier. These slides contain five serially sectioned and stained tadpoles of *Rana pipiens* experimentally infected at Douglas Lake, Michigan, with strigeid larvae and naturally infected with an unidentified gorgoderid perhaps assignable to the genus *Gorgoderina* (Fig. 5). These small worms were found in the tadpole's cloaca, urinary bladder, Wolffian ducts, and mesonephric tissue. Tadpoles are known to serve as metacercarial hosts in life cycles of other bladder flukes, but have not been reported as hosts of young, unencysted juvenile worms. Because of constancy of size and lack of development toward sexual maturity it is possible that these worms remain as juveniles until metamorphosis of their host or until the host is eaten. If the former, a condition known for the roundworm parasites, *Rhabditis maupasi,* of the common earthworm which survive indefinitely as juveniles in living earthworms (Cf. Goodchild, 1950), there still remains in doubt the method of infection of the tadpole because other adult hosts are almost invariably carnivorous; if the latter, the manner of passage of the unprotected worm through the enteron of the predacean (especially the stomach—in which the metacercarial cyst offers protection in other gorgoderids), as well as the way of infection of the tadpole, remain unanswered.

SUMMARY

1. Bladder flukes, *Gorgoderina attenuata* and *Gorgodera amplicava,* of the trematode subfamily GORGODERINAE parasitizing frogs, *Rana catesbeiana* and *R. grylio,* from widely separated regions in the continental United States, have been found regularly in the Wolffian ducts and encapsulated in the mesonephroi.

2. Cysticolous *Gorgoderina attenuata* may attain sexual maturity and reproductive effectiveness within the kidney tissue in capsules of host origin; ripe eggs containing fully-formed miracidia pass into the capsular cavity, through the mesonephric tubules, and the Wolffian duct to reach finally the outside.

3. Bladder flukes were encapsulated within the kidney by the action of granulocytes which surround the parasite initially in a single layered wall subsequently made multicellular by cell division from a cytogenous basal layer. The capsular wall is also cytomorphically altered to form a trophic layer on the internal epithelial lining. The trophic epithelium elaborated and secreted into the capsular cavity an acellular, granular, mucoid-like material which also was invariably present in the caeca of the parasite.

4. Adult worms recovered from capsules were markedly different in behavior and appearance from specimens of the same species residing in the urinary bladder; kidney forms were smaller, weaker, more transparent and more attenuated.

5. Massive gorgoderid infection in the mesonephroi, mesonephric tubules, and Wolffian ducts was detrimental to the frog host and perhaps was even a cause of death. These parasites may, therefore, have economic importance.

6. Tadpoles of *Rana pipiens* (from the northern Michigan region) have been found infected with juvenile gorgoderid trematodes. Worms were recovered from the cloaca, the urinary bladder, the Wolffian ducts, and the mesonephroi of the tadpole.

Key to Abbreviations Used in the Illustrations

AT	Anterior testis	LM	Longitudinal muscle
B	Brain	M	Mouth
C	Caecum	MI	Miracidia in unhatched eggs
CC	Cuboidal cell	MS	Mucoid-like material
CL	Capsular lumen	MT	Mesonephric tubule
CM	Circular muscle	O	Ovary
CSC	Columnar secretory cell	OM	Oblique muscle
CU	Cuticula	OS	Oral sucker
CW	Capsular wall	P	Parenchyma
EC	Erythrocyte	PC	Polyhedral cell
EG	Esophageal gland	PT	Posterior testis
EP	Excretory pore	SV	Seminal vesicle
F	Fibroblast	TU	Terminal uterus
FL	Fibrous layer	U	Uterus
G	Granulocyte	VG	Vitelline gland
GP	Genital pore	VS	Ventral sucker
L	Lacuna		

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Explanation of Plate 1

All figures were made with the aid of a camera lucida or projection apparatus to the following scales: A scale—1.0 mm.; B scale—0.5 mm.; C scale—0.2 mm.; D scale—0.15 mm.; E scale—0.05 mm.

Fig. 1. *Gorgoderina attenuata,* whole specimen from the urinary bladder of *Rana catesbeiana.*

Fig. 2. *Gorgoderina attenuata,* whole specimen from a capsule in the kidney of *Rana catesbeiana.*

Fig. 3. *Gorgoderina attenuata,* whole specimen from a capsule in the kidney of *Rana catesbeiana.*

Fig. 4. *Gorgoderina attenuata,* near median sagittal section, in a capsule in the kidney of *Rana catesbeiana.*

Fig. 5. *Gorgoderina* (?), immature whole specimen from the Wolffian duct of a tadpole of *Rana pipiens.*

PLATE I

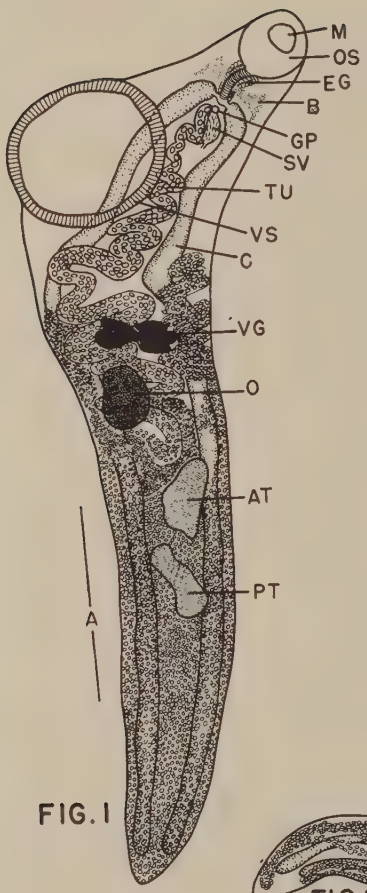

FIG. 1

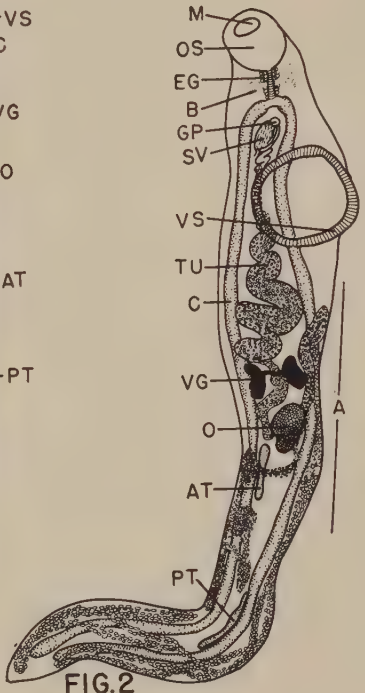

FIG. 2

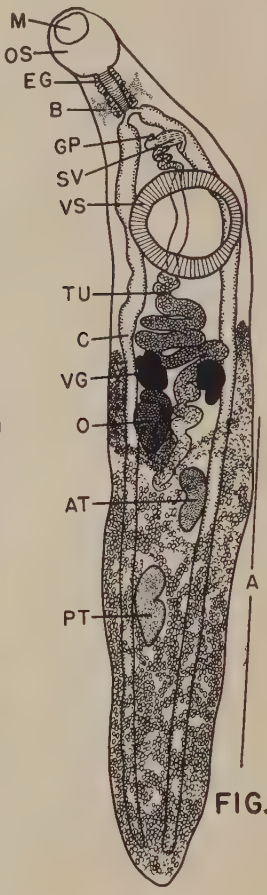

FIG. 3

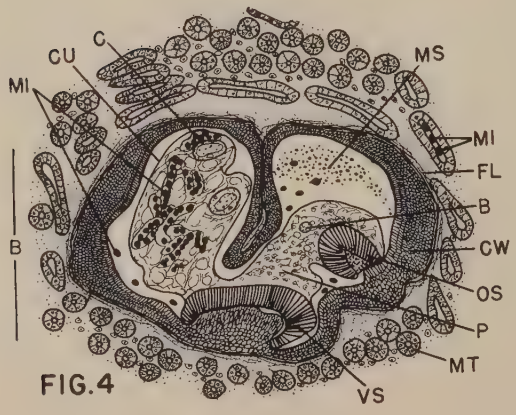

FIG. 4

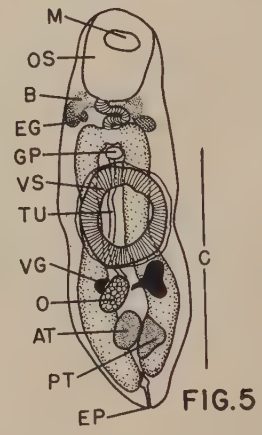

FIG. 5

PLATE 2

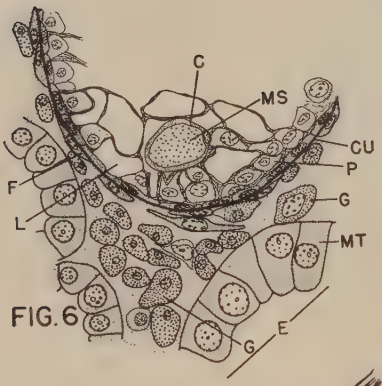

FIG. 6

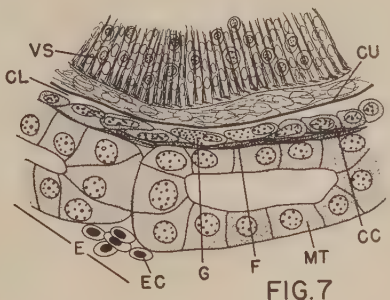

FIG. 7

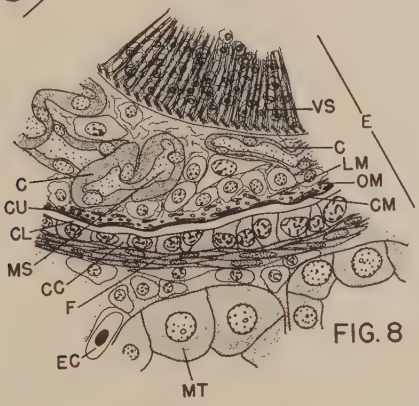

FIG. 8

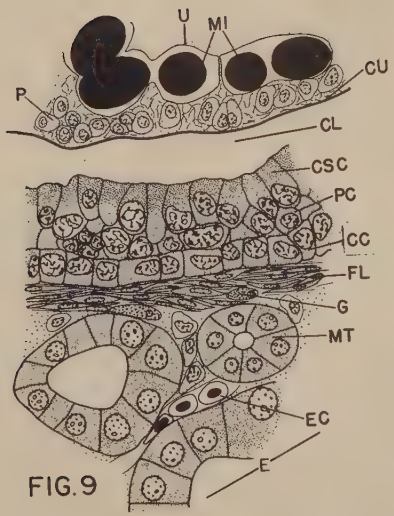

FIG. 9

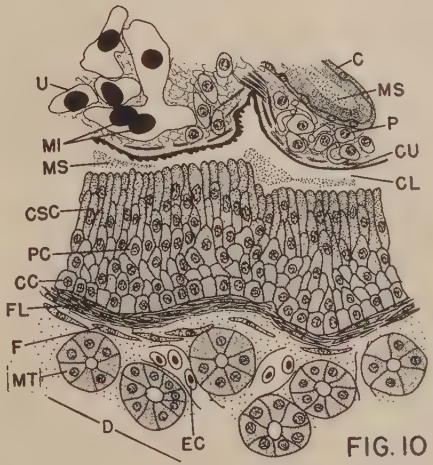

FIG. 10

Explanation of Plate 2

All figures on this plate show only small sections of the wall of *Gorgoderina attenuata* and adjacent host tissue. In each illustration the tissue of the parasite is above, the tissue of the host below. Figures were made with the aid of a camera lucida or projection apparatus to the scales given under explanation of Plate 1.

Fig. 6. Details of early encirclement of young parasite with connective tissue cells of the host.

Fig. 7. Formation of a unicellular layer of host cells around the parasite; cellular differentiation is also depicted.

Fig. 8. Details of the capsular wall showing the simple cuboidal epithelium and the fibrous layer.

Fig. 9. Augmentation of capsular wall to form a multicellular layer around a sexually mature parasite.

Fig. 10. The fully-formed multicellular capsular wall around a sexually-mature parasite.

SNAIL PONDS

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In 1930 this author began snail studies for the Field Sanitary Engineering Section of The Rockefeller Foundation in Egypt.

Close observation of snails in numbers large enough to secure accurate data presented one of the greatest difficulties.

Rearing snails in aquaria lifts them up out of their natural environment of darkness on all sides but one into an unnatural condition of light from all sides but one. The unnatural glass surface replaced the comfortable mud surfaces of the canals, drains and ponds. But possible observation of daily activities is the main factor gained and this is no small gain.

Accurate control over the snail population with separation methods essential to numerical studies in breeding and infection, as well as in life span, are more easily obtainable in glass aquaria.

But study of long-time activities not particularly connected with numerical data are best made in the natural environment. It is apparent that this is encompassed with difficulties. The establishment in 1934 of an artificial environment which is so nearly like the natural as to reproduce it has been the aim of this author's experimental ponds which are located just outside of the laboratory windows, in Cairo, to facilitate ease of observation. As time has passed they have become even more environmental.

In cases where it is desired to infect large numbers of snails, one or more ponds can be used to accommodate 10 to 50,000 snails during exposure to miracidia, and, with careful feeding and continuous water flow, these large numbers can be reared to shedding of cercariae. This is important in cases where infected snails are requested for shipment abroad or for furnishing cercariae for antigen.

A short description of the construction of these canals and ponds may be of use to prospective builders.

The ditches and cavities are dug deeply enough to accommodate a good foundation and the bottom is soaked with weed-killer. Coarse gravel and cement 5 to 1 is tamped into place, followed by sand and cement 2 to 1. A top dressing of fine sand and cement in equal parts is finally applied. When dry enough to hold water the pond is filled, the water is left four days and then drained off. This process is repeated three times. Then mud is spread over the surface and acidulated water run slowly till full. This should stand two to three weeks. Then the mud and water are removed and the surface brushed with a wire brush and clean water. This is run off and the canals are ready to fill for use. Silt water is first run in and allowed to settle and then the water is run off and the silt planted to the water plants desired. The ponds and canals are filled with water, and fish (*Gambusia* and *Telapi*) are put

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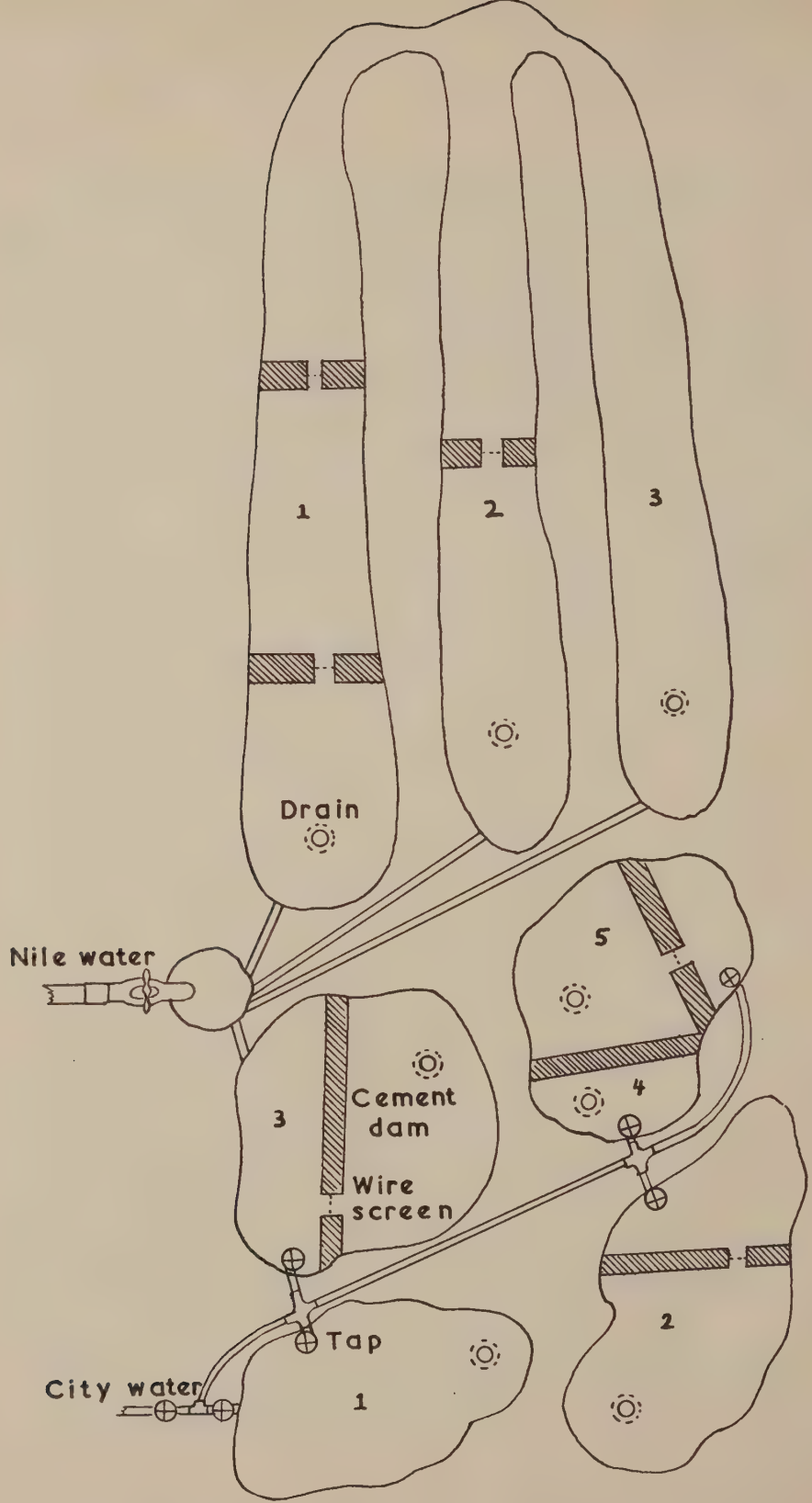


LABORATORY SNAIL PONDS & CANALS

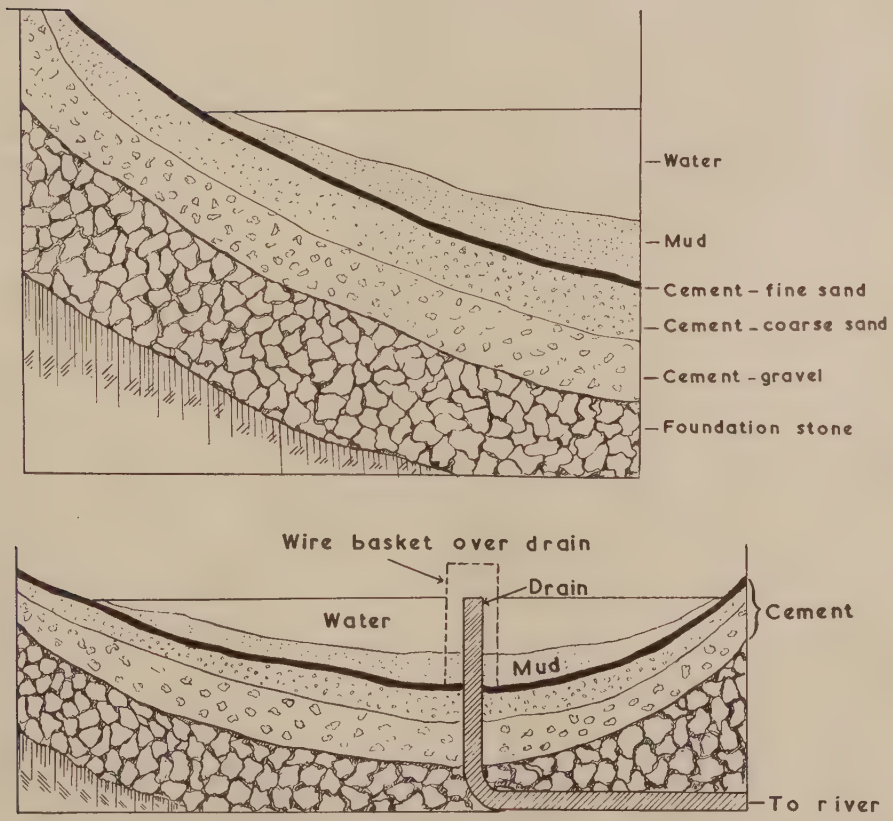
in to keep down mosquitoes. Frogs and toads immediately take up residence, water insects begin to come and *Physa* makes its appearance.

If the ponds are not matured before using them for snail rearing, the lime in the water kills the snails.

The sides of the canals conform to the shape of those commonly existing in the environment and they are joined in such a way as to get the greatest length into available space. The ponds are shaped to meet special needs, such as depth and shallows, slopes toward or away from the sunlight and divisions to make separations easy and convenient.

Convenient drainage is arranged so that each or all canals can be fully drained at will and a constant overflow established for the accommodation of running water.

CROSS SECTION of POND



The mud water proportion is such that plants like water lily (*Nymphaea*) flourish and seed down year after year, and rushes (*Cyperus*) and cattail flags (*Typha*) grow luxuriantly.

The plankton of these ponds and canals is exactly like that of environmental

canals and the insect, fish and animal life parallels that of the environment except that the species are deliberately selected. Various water-loving birds frequent the ponds.

In countries too cold to rear snails out of doors it would be possible to pipe the sides of the canals and ponds with hot-water pipes and so keep the water at a favorable temperature.

For tropical and subtropical snails a glazed shed could be arranged to cover the ponds in winter.

The environmental ponds vary in size from 9 m. to 14 m. in circumference. The water is from 20 cm. to 30 cm. deep and the mud is from 5 cm. to 8 cm. deep and is silt deposited from the Nile water as occurs in small ponds in the country. The canals are from 13 m. to 15 m. long by 1.80 m. to 2 m. wide. They vary in depth from 10 cm. to 55 cm.; the water and mud in shallow to deep places runs from 4 cm. water and 6 cm. mud to 40 cm. water and 15 cm. mud.

The mud is deposited naturally from the continuous supply of Nile water just as it is in the environment. Algae and water plants sometimes become too numerous and must be removed to prevent overstocking and crowding out snails.

The canals 1, 2, and 3 can be connected into single or combined canals thus giving greater or less length as desired.

Of the ponds 1, 2, 3, 4, and 5, some are shallow and some deep, and some are divided by screened dams.

Canals vary in depth at one end or the other.

THE STANDARDIZATION OF FECAL SMEARS FOR ESTIMATING EGG PRODUCTION AND WORM BURDEN[1]

PAUL C. BEAVER[2]

It has been shown that estimates of hookworm burden can be based on egg counts in direct fecal smears that are made to a uniform density (turbidity) by means of a photoelectric light meter (Beaver, 1949). As originally described, the method is lacking in two respects. Because photoelectric light meters are manufactured in various models and patterns and, therefore, require diverse types of adapters, the standardization of smears requires a reliable method of calibrating the meter assembly; and since the meter may deteriorate or become damaged, some means of checking its accuracy from time to time is needed. The other obstacle to the general application and usefulness of the method is the lack of standardized smears offering a range of densities suitable for diverse needs and preferences. Both problems are dealt with in this study.

MATERIALS AND METHODS

Stools bearing hookworm eggs were not available, whereas those containing eggs of *Ascaris lumbricoides* and *Trichuris trichiura* were easily obtainable. It was necessary, therefore, to test the reliability of direct smear egg counts on these two species, especially the latter because of its more posterior location in the intestine. Following these preliminary studies egg counts were made on a series of smears both more dense and less dense than the original standard to determine the quantities of feces in smears of various densities. From the above, the densities of smears containing 1/200, 1/300, 1/400, 1/500 and 1/1,000 cc. of feces were determined and these findings were confirmed by counts on an additional series of stools. Finally, barium sulfate suspensions with densities equal to each of the above five fecal smears were produced and described.

The apparatus used in making the standard fecal smears consists of a photoelectric foot-candle meter with the window of its cell reduced in diameter by fitting to it a block about 15 mm. thick bearing a central hole 16 mm. in diameter. A lamp which is made adjustable in height is suspended directly above the meter window. The standard fecal smear is made as follows: A clean microscope slide is placed over the window and the light is adjusted to give an arbitrarily chosen reading, 20 foot-candles for example; a drop (0.05 cc.) of water is placed on the slide, adjusted over the window, spread just to the window's edge, and enough feces is added to reduce the light to one-half its original reading, to 10 foot-candles.

Normal mushy or mushy-formed stools from children four to twelve years of age were used. The entire series of smears required from a particular stool or from a particular dilution flask were made one immediately after the other and were allowed to dry. The counting was then carried out at convenient periods, each smear being covered with one or two drops of cedar oil just prior to the counting.

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[2] With the technical assistance of Mrs. Beatrice Madril.

A coverglass was not used. By this technic *Ascaris* and *Trichuris* eggs are conspicuous while most other elements of the smear are cleared so that counts can be made rapidly and accurately with low magnification. In addition to being clear, the smears are only about 16 mm. in diameter and present a thin flat microscopic field. This use of cedar oil to clear the dry smears is a modification of Hein's (1927) technic for finding helminth eggs in feces. The technic is unsuitable for hookworm eggs. Dilution counts were made by the Stoll and Hausheer (1926) modification of the Stoll (1923) method.

RESULTS

Reliability of direct smear and dilution egg counts.

Statistical analysis of ten dilution counts and ten direct smear counts on each of ten stools containing *Ascaris* and a like number containing *Trichuris* eggs, showed that the reliability of egg counts, as measured by their variability, was essentially the same by the two methods (Table 1).

TABLE 1.—*Summary of egg counts made to determine the reliability (coefficients of variation) of the dilution and direct smear methods on stools containing* Ascaris *and* Trichuris *eggs, and to determine the quantitative relationship between them*

Stool	Dilution counts*		Smear counts*		Thousands eggs/cc.	
					by dilution	by smear
	eggs/ slide	coef. of variation	eggs/ slide	coef. of variation	corrected by density	eggs/slide × 300
A-1	376	9.2	384	10.0	150	115
A-2	184	8.6	111	11.7	37	33
A-3	110	6.5	73	15.9	28	22
A-4	127	8.7	105	20.1	25	32
A-5	5	47.5	20	30.9	5	6
A-6	44	17.8	20	37.3	7	6
A-7	29	29.1	18	20.0	4	5
A-8	79	8.5	45	15.2	16	14
A-9	36	11.8	42	11.3	18	13
A-10	51	13.9	42	20.4	15	13
Ascaris ave.	104	16.2	86	19.3	30	26
T-1	217	14.3	360	9.9	87	108
T-2	13	24.0	35	21.7	13	10
T-3	19	23.1	16	21.7	5	5
T-4	19	15.8	11	35.3	4	3
T-5	11	18.5	10	30.2	3	3
T-6	13	30.4	21	16.5	5	6
T-7	5	45.1	5	34.3	2	2
T-8	15	25.8	14	31.4	2	4
T-9	26	16.6	16	20.5	5	5
T-10	133	6.2	127	10.4	33	38
Trich. ave.	47	22.0	62	23.2	16	18
20 stool ave.	75	19.1	74	21.2	23	22

* Average of counts on 10 smears from each stool.

Quantitative relationship between dilution and direct smear counts.

In the earlier study, which was made on hookworm infections, dilution egg counts in terms of eggs per cc. (fsb) were found to be roughly 300 times the direct smear counts in terms of eggs per slide. This value differed greatly from stool to stool, ranging all the way from 100 to above 800. However, when dilution counts were converted to eggs per cc. on the basis of density of the suspension rather than consistency of the stool, the correlation between counts by the smear and dilution methods was found to be much closer, and the conversion factor 300 was suitable for all except the extreme types of stools.

On the present series of stools there is also considerable range in the factor relationship between corrected dilution counts in terms of eggs per cc. and the direct smear counts in terms of eggs per smear. However, when direct smear counts are converted to eggs per cc. on the basis of 300 as the conversion factor, wide differences occur in only a few of the twenty stools (Table 1).

Quantities of feces in smears of various densities.

It was found by preliminary trial that smears made heavy enough to reduce the meter reading from 20 to 6 contained approximately twice as many eggs as the original standard smear (which reduced the meter reading from 20 to 10), and very light smears made by reducing the reading from 20 down to 17 were found to contain approximately one-fourth as many eggs as the original smear. Thus smears containing amounts of feces ranging from 1/200 cc. to 1/1,000 cc. would be produced at densities between 6 and 17, the designations "6" and "17" being used to indicate smears of such density as would reduce the meter reading from 20 to 6 and from 20 to 17. On six additional stools counts were made on ten smears each at densities 6, 8, 10, 11, 12, 15, and 17. Assuming that smears made at density 10 contain 1/300 cc. of feces, the amount of feces in each of the other smears could be deter-

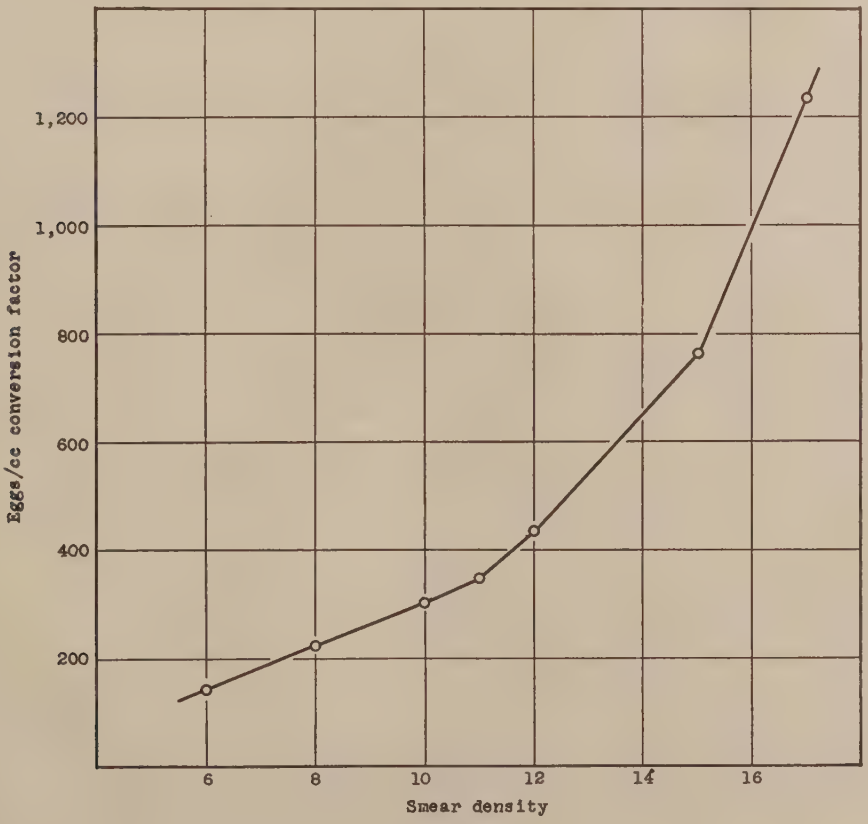

FIG. 1. Relationship between smears of various densities, based on table 2.

mined by dividing the total count at density 10 by the total count for each of the other densities and then multiplying this figure by 300. The results (shown in Table 2) indicate that counts of eggs in smears made at the seven densities 6, 8, 10, 11, 12, 15, 17 respectively would be converted to eggs per cc. by the factors 148, 224, 300, 344, 433, 763, and 1,234.

TABLE 2.—*Direct smear egg counts on 6 stools with smears made at a range of densities that were determined by preliminary trial to extend from below 1/200 to above 1/1,000 cc., i.e., from densities 6 to 17. All figures are based on the average of counts on 10 smears*

Stool	Average eggs/smear at density						
	6	8	10	11	12	15	17
AA	42	27	20	18	15	7	4
BT	10	7	5	5	4	3	1
CA	37	26	17	16	10	6	4
DT	23	16	14	12	9	5	3
EA	93	58	45	36	29	16	10
FT	32	24	16	15	14	9	4
Total	237	158	117	102	81	46	26
$\frac{117^*}{\text{total}} \times 300$	148	224	300	344	433	763	1,234

* Total of counts at density 10, which when multiplied by 300 gives eggs per cc.

The density of smears containing 1/200, 1/300, 1/400, 1/500, and 1/1,000 cc. of feces.

When values from Table 2 are plotted as shown in Figure 1, the approximate densities that will give smears containing the desired amounts of feces can be determined by interpolation and are respectively 7.5, 10, 11.5, 13, and 16 for smears containing 1/200, 1/300, 1/400, 1/500, and 1/1,000 cc.

To test these values, five additional stools were studied. On four of them ten smears each were made at densities 10, 11.5, 13, and 16, and on the other one, ten smears were made at 7.5 in addition to the above densities. The average counts at each density converted to eggs per cc. gave essentially the same values for all smears on each of the five stools (Table 3).

TABLE 3.—*The average of 10 counts of eggs per slide at densities determined on the basis of Figure 1 as giving smears containing 1/300, 1/400, 1/500 and 1/1000 cc. of formed stool and the computed eggs per cc. values*

Density	10		11.5		13		16	
Stool	EPS*	× 300	EPS	× 400	EPS	× 500	EPS	× 1,000
I A	126	37,800	72	28,800	61	30,500	28	28,000
II T	92	27,600	80	32,000	66	33,000	35	35,000
III T	127	37,100	103	41,200	79	39,500	43	43,000
IV A	58	17,400	48	19,200	33	16,500	15	15,000
V T**	155	46,500	127	50,800	98	49,000	52	52,000
Average eggs/cc.		33,280		34,400		33,700		34,600

* Eggs per slide, based on the average of 10 counts.
** At density 7.5, EPS (241) × 200 = 48,400 eggs per cc. in Stool V.

A method of calibration.

Solutions of 2N Na_2SO_4 and N/1 $BaCl_2$ were made up by analytical methods and mixed separately with pure glycerine in the proportions of 2 parts of salt solution to 1 part glycerine. Thus regardless of how the two salts were later mixed to give barium sulfate precipitate, the mixture contained the same proportion of glycerine to hold the barium sulfate temporarily in suspension. The two salt-glycerine mix-

tures were combined in various proportions to give a wide range of concentrations at numerous close intervals, the barium chloride solution always being added to the sodium sulfate solution slowly, drop by drop, with constant stirring. The turbidity of each suspension was measured on the adapted light meter in the same manner as fecal smears, one drop from a pipette that delivered 0.05 cc. of distilled water. This was repeated numerous times at varied intervals for a period of 6 months for each suspension and several sets of dilutions were made up and tested in like manner. It was found that after a few hours the density of each suspension remained constant.

From the data in Figure 1 and Table 3 it was shown that smears containing 1/200, 1/300, 1/400, 1/500, and 1/1,000 cc. respectively have densities of 7.5, 10, 11.5, 13, and 16. The barium sulfate suspensions which give the above densities are shown in Table 4.

TABLE 4.—*The amounts of 2N Na_2SO_4 and N/1 $BaCl_2$ (each mixed with ½ part of pure glycerine) necessary for making suspensions of $BaSO_4$, 0.05 cc. (one drop) of which will give density readings equal to smears containing specified quantities of feces*

Parts barium chloride mixture	Parts sodium sulfate mixture	give barium sulfate suspension for standardization of smears containing feces in the amount of
3	2	1/200 cc.
2	3	1/300 cc.
1	2	1/400 cc.
1	3	1/500 cc.
1	6	1/1,000 cc.

To calibrate a photoelectric light meter for making smears of a standard density the meter would first be fitted with a mask to reduce its window to about 16 mm. in diameter and an adjustable light would be suspended directly above it. A series of the barium sulfate standard suspensions would be prepared and with a pipette that delivers 0.05 cc. of distilled water a drop of one of them would be placed on a clean slide and tested as follows: Place the slide on the meter with a clear portion over the window; adjust the light to bring the indicator hand to any whole number with the light at a convenient distance from the slide; move the slide to bring the drop of standard suspension over the window and spread the smear just to the window's edge; by moving the smear away and returning it to the window the amount of light reduction produced by it can be determined. After repeating the procedure several times constant results will be obtained and the calibration for fecal smears of that particular density will thus be accomplished. With the same set-up, fecal smears in one drop of water will be made just dense enough to produce the same amount of light reduction as the standard barium sulfate suspension.

DISCUSSION

The egg-worm ratio for direct smear counts can be determined for other species as it has already for the hookworm, *Necator americanus* (Beaver, 1949), and the relative worm burden can be measured reliably even without this knowledge. Thus for many purposes the utility of the direct smear method does not depend upon the interpretation of its counts in terms of eggs per unit of feces. On the other hand, without the possibility of making reference to and comparison with egg count data recorded as eggs per cc., the usefulness of direct smear counts would be definitely restricted. All direct smear counts, therefore, should be convertible, roughly at least,

to eggs per cc. Probably there is no advantage in attempting to reach a high degree of accuracy in determining conversion factors because an egg count by any method is a statistic with considerable error in the estimate. On the other hand, it is important to have the direct smear method standardized so that each count is highly reliable as an estimate of the mean direct smear count on that particular stool. At the present time the only common expression for direct smear counts of different densities, containing various but standard quantities of feces, is eggs per cc. Estimates of eggs per cc. by the direct smear method will not always be the same as estimates on the same stool by the dilution method. On the average, however, as an estimate of worm burden, direct smear egg counts in terms of eggs per cc. will be as reliable as dilution counts expressed in the same terms.

For most routine counts, smears containing 1/500 cc. are most useful. For stools containing very large numbers of eggs or for counting small inconspicuous eggs, the 1/1,000 cc. smears are better. If the modified Hein technic is being used and the eggs are scanty there is considerable advantage in using the 1/200 or 1/300 cc. smears.

SUMMARY

Egg counts on either *Ascaris lumbricoides* or *Trichuris trichiura* are about equally variable, and presumably equally reliable, by the standard direct smear (Beaver, 1949) and dilution methods (Stoll, 1923).

By comparison with the original standard smear the quantities of feces in smears of various desnsities ranging from very heavy to very light were found, and from these data the densities of smears containing 1/200, 1/300, 1/400, 1/500, and 1/1,000 cc. of feces were determined.

A barium sulfate suspension, one drop (0.05 cc.) of which has the same density (turbidity) as the fecal smear, was described for each of the five standard smears.

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ON A MYXOSPORIDIAN (PROTOZOAN) PARASITE OF CALIFORNIA TROUT

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During the summer of 1948 Mr. J. H. Wales, District Fisheries Biologist of the Division of Fish and Game, Department of Natural Resources of California, sent me specimens of fingerling Rainbow trout which were heavily infected with a myxosporidian parasite. Mr. Wales stated that the parasite caused considerable loss among trout in the Crystal Lake Hatchery at Mount Shasta, California. I shall briefly mention the organs infected in the fish sent to me, and describe the parasite. Measurements were made of fixed and stained material. Diagnostic characteristics of the parasite are as follows (see Plate I):

Specific diagnosis: Genus *Ceratomyxa*, species *shasta n. sp.*
Trophozoite rounded or widely variable in shape. Sporoblasts each containing two spores which average 12.7 by 19.0 microns. Ectoplasm not distinct, endoplasm lightly granular. Each sporoblast generally contains two spores, occasionally four. One of two residual nuclei visible in mature sporoblasts ready for sporulation.
Spores 6 by 14 microns with broadly rounded ends which are reflected posteriorly. Many individuals more strongly arched than any previously described in the genus. No striations on shell valves. Suture line straight, raised and distinct. Polar capsules 8 microns in diameter and close together at anterior end. Sporoplasm fills spore and contains 2 nuclei.
Host: Salmo gairdnerii Richardson.
Location: Widely distributed in viscera.
Locality: Crystal Lake Hatchery, Mount Shasta, California.

This is the first report of the genus *Ceratomyxa* in a fresh water fish, and the first report of the genus as a tissue parasite. Species of *Ceratomyxa* occur widely in marine fishes where they infect the lumen of gall and urinary bladders. Organs which I found to be infected in three fingerling trout are:

Stomach: in serosa, longitudinal and circular muscles, and especially in connective tissue between these muscles.

Intestine: all layers, often so heavily infected that layers are hardly recognizable.

Esophagus: generally in all layers, especially mucosa and submucosa.

Spleen: cortex only.

Skin: subcutaneous connective tissue.

Gills: light infection in capillaries.

Liver: outer connective tissue layer in light infections; throughout all liver tissue in heavy infections. Much destruction of tissue.

Kidney: in and around uriniferous tubules, especially within the tubule cells. Much destruction of tissue.

Heart: none in muscle cells, few in lumen.

Gall bladder: light infection in outer layer.

Gonads: light infection in and under outer layer.

The following list of other species of trout infected with myxosporidia, together with the parasites and organs invaded, has been compiled from the literature.

Received for publication, March 27, 1950.

Host	*Parasite*	*Organs invaded*
Salmo clarkii	*Myxidium sp.* Davis 1947	kidney tubules
Salmo fontinalis	*Lentospora cerebralis* Plehn 1905	cartilage, perichondrium
Salmo salvelinus	*Henneguya salvelini* Zandt, 1923	subcutaneous tissue
Salvelinus fontinalis	*Zschokkella salvelini* Fantham, Porter & Richardson 1939	kidney surface
	Myxobolus ovoidalis Fantham 1930	skin
	Henneguya fontinalis Fantham, Porter & Richardson 1939	skin
	Chloromyxum truttae Léger 1906 (reported from *S. fontinalis* by Davis 1947)	gall bladder
	Chloromyxum leygidi Mingazzini 1890 (reported from *S. fontinalis* by Fantham and Porter 1947)	gall bladder
Salvelinus kundscha	*Chloromyxum salvelini* Fugita 1923	gall bladder
Trutta fario	*Chloromyxum truttae* Léger 1906	gall bladder, gall duct
	Myxobolus neurobius Schuberg and Schröder 1905	nervous tissue
	Myxidium sp. Gauthier, 1926	gall bladder
	Myxidium truttae Léger, 1930	gall bladder
Trutta iridea	*Lentospora cerebralis* Plehn, 1905	cartilage, perichondrium
Trutta salar	*Myxidium oviforme* Parisi, 1912	gall bladder
	Lentospora cerebrales Plehn, 1905	cartilage, perichondrium

The new infection described in this paper is considerably more extensive than in any previously reported case from trout. Details of this infection are being assembled for publication by Mr. Wales. More recent information from Mr. Harold Wolf, Parasitologist at Crystal Lake Hatchery, reveals that the outbreak in the summer of 1949 was heavier than that during the previous summer, and that by the end of September the mortality among fingerling fish was one hundred per cent. Death of the fish was presumably caused by the myxosporidian parasites, but conclusive evidence was not presented.

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PLATE I

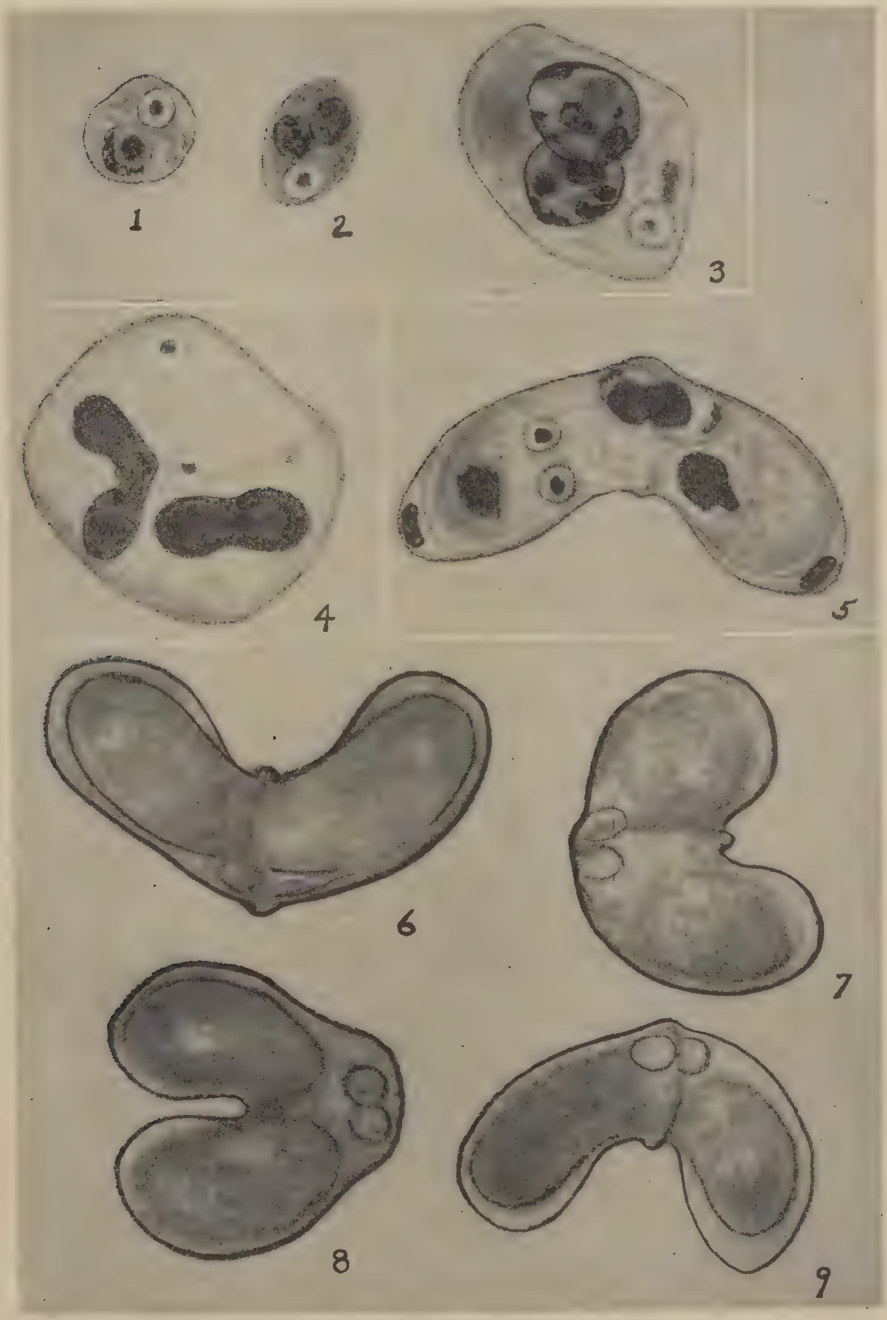

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PLATE I

All figures are of *Ceratomyxa shasta n. sp.* Figures 1–5 fixed in formalin and stained with Heidenhains iron hematoxylin. Figures 1–4, ×2400; 5–9 ×3800.

FIG. 1. Young trophozoite with two nuclei, one of which (light colored) is the residual nucleus.

FIG. 2. Older trophozoite with one residual nucleus and two darker nuclei destined to become spores.

FIG. 3. Sporoblast with two young spores.

FIG. 4. Sporoblast with two maturing spores and two residual nuclei.

FIG. 5. Mature spore showing the eight nuclei typical of spores of all myxosporidia.

FIGS. 6–9. Mature spores from formalin fixed, but unstained host tissue.

LABORATORY REARING METHODS FOR THREE COMMON SPECIES OF BIRD MITES

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In connection with virus transmission studies now in progress, living bird mites were required in various stages of development. To fulfill this need, colonies of *Liponyssus sylviarum* Canestrini and Fanzago and *Dermanyssus gallinae* (De Geer) were established from specimens collected in Kansas and Colorado by Mr. Virgil Miles of the Midwestern CDC Services. Specimens for starting a colony of *Liponyssus bursa* (Berlese) were obtained in the vicinity of Montgomery, Ala. by the authors.

No reference has been found regarding laboratory colonization of either *L. bursa* or *L. sylviarum,* but several workers have reported on methods for colonizing the related tropical rat mite, *L. bacoti* (Hirst). Noteworthy among these investigators are Bertram *et al* (1946) and Williams (1946), who described the use of rearing chambers placed in pans of water or oil to prevent the mites from escaping, and Scott *et al* (1947), who designed a sheet metal chamber provided with a moat at the top for the same purpose.

Dermanyssus gallinae has been reared successfully by Margaret G. Smith and her coworkers at the Washington University School of Medicine, St. Louis, Mo. (personal communication) in tall metal popcorn cans surrounded by creosote. Well-fitting lids, with centers cut out and covered with fine mesh cloth, were sealed onto the cans with paraffin each time they were set in place. A chick introduced into a can one or two nights a week maintained the colony, for the specimens fed rapidly and then went into hiding under a small wooden platform provided on the bottom.

The purpose of this report is to describe in detail methods for rearing *L. bursa* and *L. sylviarum. D. gallinae* has been included because the use of similar procedures for all three species may prove more convenient from the maintenance standpoint in the laboratory.

MATERIALS AND METHODS

The rearing chamber used is a modification of the type described by Scott and his associates (1947) for maintaining *L. bacoti* (Hirst), and, except for slight alteration, is similar to that used at the Technical Development Services of the U. S. Public Health Service, Communicable Disease Center, at Savannah, Ga. for the same species. It is a sheet-metal can 12 inches wide, 15 inches long, and 17½ inches high, surrounded at the top by a moat 1½ inches deep and 1 inch wide, the outer rim of which projects ½ inch higher than the inner rim of the can (Fig. 1). This greater elevation of the outer rim allows placement of a sheet-metal lid to keep out flying insects and to reduce the amount of light entering the rearing chamber. The lid is provided with a handle in the center and has two 4- by 10-inch cut-out sections covered with 40 mesh screen. All seams of the can are soldered watertight to prevent escape of mites.

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[1] From the Entomology Laboratory, Virus and Rickettsia Section, Montgomery, Ala.

Dried grass, autoclaved to prevent contamination of the colony with unwanted arthropods, is packed in the bottom of the can to a depth of about 3 inches. Both Bermuda grass and Johnson grass have been used with success, and probably any type which does not mat too tightly would prove satisfactory. A sheet-metal tray is attached on each end of the can with adhesive tape at a height which allows its bottom to rest on the grass (Fig. 2). One tray is used for water and the other for sterilized chick mash. These trays are $8\frac{1}{2}$ inches long, $1\frac{3}{4}$ inches deep, $1\frac{1}{2}$ inches wide at the bottom, and $1\frac{1}{4}$ inches wide at the top, dimensions which quite successfully

FIG. 1. Sheet-metal chamber for rearing bird mites, full view.

discourage perching by young chicks. The back of each container extends $1\frac{1}{2}$ inches higher than the remainder to facilitate taping to the end of the can.

To start a colony, a chick less than a week old is placed in the can, $\frac{1}{4}$ inch of purified mineral oil or $\frac{3}{4}$ inch of distilled water put into the moat, and several hundred to several thousand mites introduced. Purified mineral oil is used, as it does not give off fumes toxic to mites. Tap water was found to be less satisfactory than distilled water, as a scum and precipitate tended to form, probably due to reaction between the dissolved salts and the galvanized metal.

For handling the mites, an aspirator (Fig. 3), similar to a type developed and used by Dr. Harold R. Dodge (personal communication) for many years, is very useful. It is so easily constructed that numbers of them may be prepared in advance and a clean one used for each mite transfer. The mite-containing portion may be

pulled apart quickly and dropped into a can to "seed" a colony. With the tip properly sealed, such an aspirator will serve also for storage of mites.

D. gallinae is the most easily maintained as it feeds quickly and then goes into hiding. Two or three pieces of corrugated cardboard, about 4 by 6 inches in size, wedged between the grass and the sides of the can, provide suitable cracks in which to hide and oviposit. A chick may be left continuously in the can, or merely placed in overnight once or twice a week for feeding. When specimens are needed for

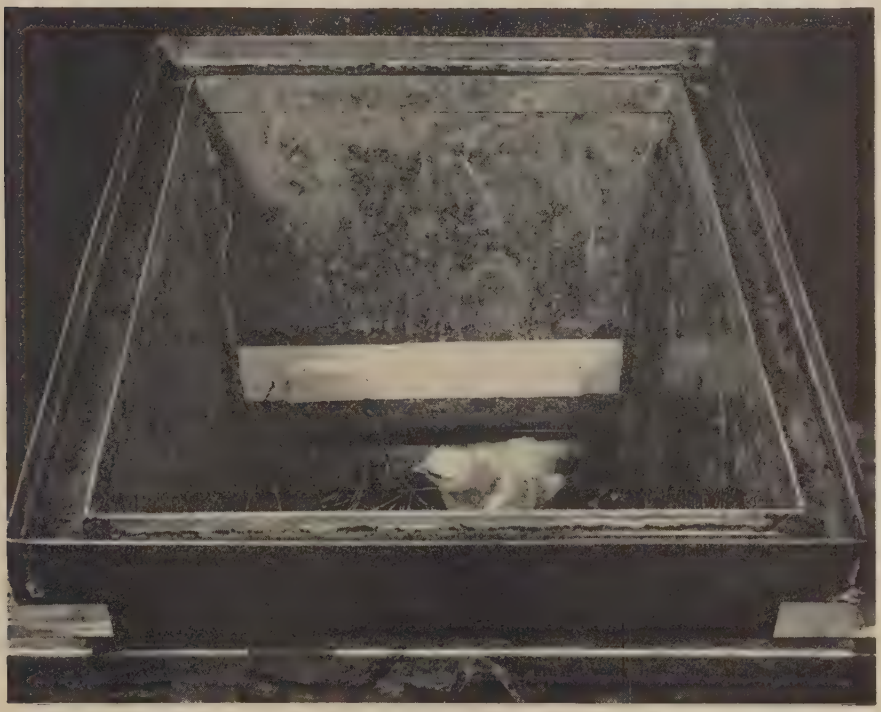

FIG. 2. View of mite rearing chamber showing watering tray, grass, chick and moat.

experimental purposes, they may be brought out of hiding by removing the chick for 3 or 4 days. They then may be aspirated from the corners and rim of the can, or one of the cardboard pieces may be moved and the mites resting on it collected.

L. bursa appears to lay most of its eggs away from the host in the litter in the rearing chamber. It is a hardy mite, apparently little affected by low humidity conditions and is easily maintained in the laboratory.

A colony of *L. bursa* was maintained in the same chamber for over 3 months and in spite of the fact that at the end of this time accumulated chick feces had transformed the appearance and texture of the grass considerably, the colony still thrived. It was then discarded, not because the filth was inimicable to survival of mites, but because *Tribolium* beetles had gained access and were breeding in large numbers. In our experience, it has proven most satisfactory to keep two or three colonies going, so that one may be discarded and one set up anew each month.

While no attempt has been made to start colonies with only one or two engorged

mites, as few as 300 protonymphs have been used. These have multiplied to inestimably large numbers within two generations. Generally, however, a thousand or more adult specimens are used. These are easily aspirated from the sides and top of an established colony. If the population of a colony reaches such a high level that chicks die of exsanguination, it may be left unfed for a few days or a week to allow reduction in numbers to occur, or several thousand mites may be removed periodically by aspiration.

L. sylviarum, while apparently having a breeding potential about the equal of *L. bursa,* is more difficult to maintain in the laboratory. Oviposition appears to occur to a large extent on the host chick and the humidity requirements of the

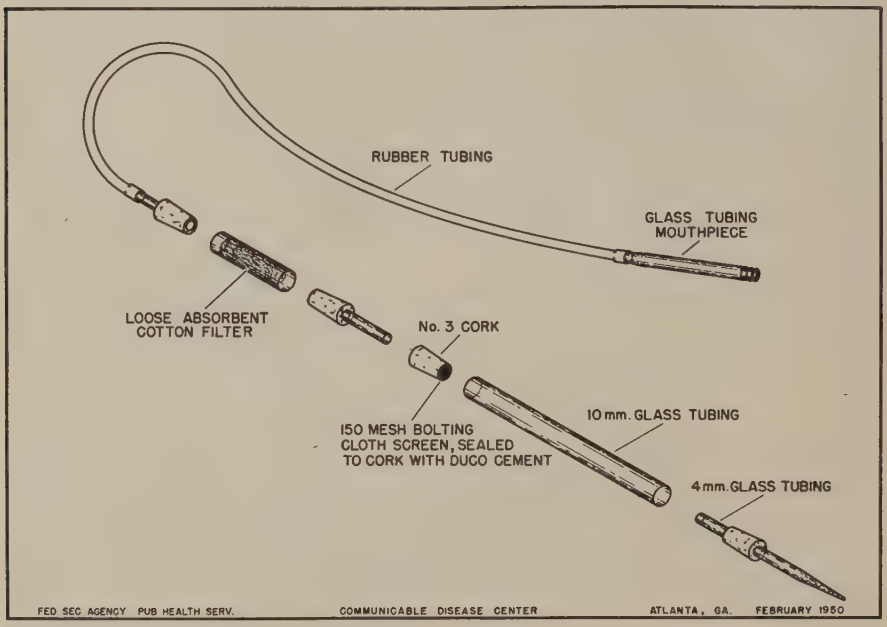

FIG. 3. Aspirator convenient for collecting and transferring mites.

rearing chamber are more exacting. In establishing a colony, one or two thousand mites are generally used to "seed" it and one or two chicks, about 2 or 3 days old, are placed in the can. Two chicks are used if difficulty is experienced in keeping them alive, because a large number of eggs and immature stages of mites are lost when a chick dies and must be removed from the colony. After the chicks are about a week old, one may be removed to prevent undue contamination of the chamber. They must be replaced, when about 15 days old, with young ones or an excess of wet feces may make conditions unsuitable for the mites; this is often true in colonies of *D. gallinae* and *L. bursa* as well as in those of *L. sylviarum.* Older chicks also may cause trouble by jumping or flying to the top of the can, hitting the lid, and thus permitting escape of mites.

With a little experience one can easily determine how moist the litter should be in a *L. sylviarum* colony. The grass should not become so dry that it crackles, nor should it be so wet as to feel actually moist. Sprinkling a few drops of water on the

grass when it appears to need it remedies dryness. Too wet grass usually is a sign that chicks have become too large, or that they have developed a diarrheal condition. As the grass mats and packs down, more may be added on top of the old to permit chicks to reach the feeders.

Although mineral oil in the moat seems to be satisfactory for retaining colonies of *D. gallinae* and *L. bursa,* it has proven destructive to colonies of *L. sylviarum.* This species may cluster near the rim of the rearing chamber, the surface of which becomes greasy when oil is used. Perhaps a deeper moat, with a greater distance between the surface of the oil and the top of the inner rim of the can, would alleviate this condition. No difficulty has been experienced in retaining *L. sylviarum* by a barrier of distilled water, provided it is skimmed occasionally. As an added precaution against escape of mites, the entire sheet-metal can containing the colony may be set into a large shallow pan of oil.

Adequate provisions should be made to keep the colonies at a temperature favorable for the survival of the chicks. The colonies at our laboratory are kept in a thermoregulated room maintained at 80°–83° F., a temperature which seems to be satisfactory for both the chicks and mites. If suitable temperature controls are lacking, a colony may be sustained through a cold period of short duration by placing a light bulb or desk lamp within a few inches of the side of the can. Heat on a single surface in this manner has a drying effect, however, and may be detrimental to the colony.

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CERCARIA LITTORINALINAE SP. NOV., A DERMATITIS-PRODUCING SCHISTOSOME LARVA FROM THE MARINE SNAIL, *LITTORINA PLANAXIS* PHILIPPI

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Penner (1942) pointed out that in certain marine swimming beaches or bays, marine gastropods may be infected with dermatitis-producing schistosome cercariae in numbers sufficient to be a problem. This was based on the knowledge that dermatitis-producing schistosome cercariae were found in the operculate marine gastropod, *Littorina planaxis* Philippi, collected along the rocky shores of certain of the Coronado Islands in Mexico and from Bird Rock, near La Jolla, California during 1940 and 1941. The intent of the present paper is to describe a new species of schistosome cercaria, *Cercaria littorinalinae* obtained from these and other collections of snails and to report experimental dermatitis on several human volunteers and on juvenile California brown pelicans.

Cercaria littorinalinae n. sp.

(Fig. 1)

Specific diagnosis: Schistosome cercaria. Apharyngeal with pigmented eyespots. Body, tail-stem and furcae uniformly spined. Average measurements[1] of 50 formalin-fixed specimens: Body length 243–312 microns, mean 271 ± 5.3 microns; body width 70–94 microns, mean 79 ± 1.9 microns; length of head organ 53–70 microns, mean 60 ± 0.6 microns; tail-stem length 245–311, mean 288 ± 9 microns; tail-stem width 28–49, mean 36 ± 1.3 microns; furcal length 137–175 microns, mean 157 ± 4 microns; furcal width 14–21 microns, mean 17 ± 0.7 microns; ventral sucker width 20–31 microns, mean 23 ± 0.8 microns; distance from posterior end of body to middle of ventral sucker 73–109 microns, mean 83 ± 3 microns. Dorso-ventral furcal finfold present. Five pairs of penetration glands in emerged cercariae with five pairs of ducts leading forward and opening through two groups of five sharply pointed penetration spines at anterior end of body. A pair of escape glands present in cercariae dissected from snail host. Digestive system a long tubular esophagus bifurcating just in front of the ventral sucker into two short ceca. Excretory system with seven pairs of flame cells, 2[(3) + (3+1)], six in body with three anterior, three posterior to ventral sucker and one in anterior portion of tail stem. An anterior and posterior collecting tubule from each side join a single collecting tubule on a level with the middle of the ventral sucker. Bladder arms form two loops, each loop with a ciliated area, close to collecting tubule junctions.

Host: Littorina planaxis Philippi[2]

Locality: Coronado Islands, Mexico; Bird Rock, San Diego County, California, U.S.A.

Of those schistosome cercariae which have been described with seven pairs of flame cells, *Cercaria littorinalinae* is different from all but two in having a markedly

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[1] Talbot method (Talbot, 1936) but sea water used as a diluent. Measurements of 50 specimens. Extremes and probable error of the mean are used to indicate the character of the variation.

[2] Determined by Dr. Joshua L. Baily.

FIG. 1. *Cercaria littorinalinae* sp. nov.

shorter tail stem. One of these, the cercaria of *Bilharziella polonica* Kowalewski (Szidat, 1929) has a smaller body and slightly shorter tail stem but a greater head-organ length and a relatively shorter distance from the ventral sucker to the posterior margin of the body. The cercariae of *B. polonica* and *G. gyrauli* are both reported from non-operculate fresh-water snails whereas *C. littorinalinae* develops in a marine operculate snail. These differences are shown in the table.

Comparison of measurements in millimeters of C. littorinalinae, C. polonica, *and* C. gyrauli

	C. littorinalinae Average of 50 specimens	*C. polonica* Szidat, 1929	*C. gyrauli* Brackett, 1940
Length of body	0.271	0.22	0.215
Width of body	0.079	0.065	0.073
Length of head organ	0.060	0.07	0.077
Length of tail stem	0.288	0.27	0.298
Length of furca	0.157	0.1	0.135
Diameter of ventral sucker	0.023	0.025	0.028
Distance from ventral sucker to posterior end of body	0.083	0.065	0.073

Several other species of schistosome cercariae with 7 pairs of flame cells have somewhat similar body measurements but differ in that they have much longer tail stems. For example, *C. parocellata* Johnston and Simpson, 1939, has a body length of 0.282 mm. but a tail-stem length of 0.362 mm. *Cercaria neocellata* Szidat, 1942, *C. physellae* Talbot, 1936, *C. stagnicolae* Talbot, 1936, and *C. dermolestes* McLeod, 1940, have body lengths of 0.27, 0.265, 0.26, 0.226 mm. respectively, whereas their tail stems are 0.39, 0.374, 0.400, and 0.340 mm. and while the body length of *C. littorinalinae* is 0.271 mm. its average tail-stem length is 0.288 mm. with a range never over 0.312 mm. Other members of the *ocellata* group are in general much larger and have markedly longer tail stems. Among these are *C. elvae* Miller, 1923, *C. pseudocellata* Szidat 1933, *C. oregonensis* MacFarlane and Macy, 1946, and *C. parocellata* Szidat, 1942 (this name cannot stand as it was used for *C. parocellata* Johnston and Simpson, 1939). There does not appear to be any possibility that the cercariae found by other authors in the *ocellata* group as discussed by Szidat (1942) in his attempt at clarifying the findings of others, or any other group for that matter, can be the same as *C. littorinalinae.*

Cercaria littorinalinae is positively phototropic and in infected snail isolation bottles of sea water, it is found clinging to the underside of the surface film within an hour or so after emergence from its host. Its position is often horizontal depending on how long it has been there after emergence. Its activity in this respect is somewhat like that of *C. gyrauli* and *C. polonica* from which it differs in several other ways. Fortunately the infected snail host makes a durable laboratory species as it can be kept dry for weeks and cercariae can be obtained for study or experimental purposes by dropping the snail into a bottle of sea water to obtain almost immediate emergence of the cercariae. This suggests that any periodicity of emergence which occurs, is governed by the incoming tides in the natural environment. A later paper will discuss more fully these and other problems connected with the snail host.

Cercarial emergence is quite interesting. If an infected snail has been kept dry for a period of a day or more, the mere act of dropping the snail in sea water will bring on the cercarial emergence. It is actually possible to watch the operculum

"unlock the door" and then to watch the shedding process. From a few to 400 cercariae may emerge at one time, often within just a few minutes, and it is most exciting to view this process with a low power dissecting microscope. Sometimes a snail can be made to shed cercariae twice a day by alternate drying and submersion with sea water, and shedding has been observed eight hours after a previous emergence in one case. Perhaps this depends on when the sporocysts break. Breaking of the sporocysts may also be the factor influencing the number of cercariae which emerge at any one time.

The use of isolation bottles is not too satisfactory for studying infections in the snail host because the snail does not stay in the container, whether it be a vial or a half-pint milk bottle. It usually crawls out as soon as it can after being placed in the sea water. When dropped into fresh water, the snails make no attempt to crawl out of the water but close their opercula tightly. No cercarial emergence takes place when the snails are placed in fresh water and cercariae placed in fresh water from crushed snails or from isolation bottles die almost at once. Infected snails remain alive in fresh water containers for a period of at least two days with their opercula closed and when removed to sea water, start crawling to the top of the container. Emergence of cercariae from such snails has been noted when the snails become active in sea water.

Infected snails kept forcibly under sea water for periods of longer than two days, are usually dead after such treatment although cercariae are often still found alive in the snail's tissues. It has not been difficult to keep infected snails alive for as long as two months stored at room temperature on dry towel paper. When weekly baths of sea water are given to the snails they will live for an even longer time. Weekly baths were accomplished by isolating the snails in individual containers or by immersing them en masse in a large aquarium for an hour or two and then returning them to the paper.

DISCUSSION

The snail host, *Littorina planaxis,* is a common inhabitant of the rocky shores along the California coast and usually lives above the mean high tide line. It is washed by the highest tides and becomes active only at these times unless it is left in a tide pool. It is especially abundant on large rocks of steep shores where bird droppings or the drainings from bird guano are common. On the Coronado Islands, Mexico, the snails are most common in and above tide pools containing considerable bird excreta, much of which comes from the breeding and roosting grounds of Western gulls, cormorants, and California brown pelicans. At Bird Rock, near La Jolla, California, the snails are especially numerous in small tide pools or on the steep walls of the "Rock" itself, usually found in or near accumulated pelican, gull, cormorant, and other bird droppings. The snails are occasionally present by the thousands, and collection usually presents no difficult problem, a small dipper, a forceps, and a quart jar being excellent equipment for the task. Although extensive collections have not been made in many areas, infected snails have been found only where abundant bird excreta exists, which suggests that one or more of the birds inhabiting the rocky shores may be the definitive host of the cercaria.

The collection and examination of 1017 *L. planaxis* 10 to 12 mm. in length from Bird Rock on March 26, 1941, revealed nearly 10 per cent of the snails infected

with mature *C. littorinalinae*. A similar number of the same size snails collected October 9, 1940, on Little Middle Coronado Islands had a 3 per cent infection rate. The percentage infection of this size group of snails has not been so great in collections made at other times of the year, and in examining over 10,000 snails, the incidence for a given year of the various sizes of snails examined from all areas has not been more than two per cent. Bird Rock collections taken at different times during the years 1940–41, and monthly collections during 1949, revealed an incidence of from less than 1 to 4.5 per cent positives from all sizes of snails examined. These observations are to be discussed more fully in another paper.

Of birds examined from the immediate areas where collections were made, only the Wyman western gull, *Larus occidentalis wymani* Dicky and Van Rossem, has been found infected with adult schistosome worms. These findings and studies of experimental nature with the cercariae indicate that the worms from the gull may be the adults of *C. littorinalinae*. Experimental life history work is now in progress.

Experimental dermatitis has been produced on human and animal volunteers. Four of six human volunteers developed schistosome dermatitis after 10–25 minute exposures to approximately 400 cercariae each. Cercariae were applied to the skin by inverting an 8 ounce isolation bottle containing the cercariae in sea water over an area on the under surface of the subject's arm. The exposed arms were not wiped off after the bottle was removed. The duration and intensity of the dermatitis in two of the cases was similar to that produced by the known swimmer's itch cercariae found in fresh water, and much milder in two others.

Approximately 1,000 cercariae were placed on the belly side of a young California sea-lion captured on the shore at Mission Beach, San Diego, California, but no later dermatitis was observed. This attempt to produce dermatitis was made because California sea-lions have been brought into the San Diego Zoological gardens from the surrounding area with a dermatitis of unknown origin which might have been produced by schistosome cercariae.

Four juvenile California brown pelicans taken from their nests were exposed to cercariae on April 22, 1941 by bathing them and pouring water containing cercariae on their more or less barren backs as well as in and on their gular pouches. Two of the pelicans had dermatitis lesions which lasted for about a week following exposure, with dermatitis most evident on April 26, 1941. At autopsy two weeks later no adult schistosome worms were found in these two birds. Two mice exposed for 35 minutes and one mouse for 10 minutes were not noticeably affected. No lesions were observed when several juvenile Western gulls were exposed to cercariae, but a Bonaparte's gull, *Larus philadelphia* (Ord), whose right foot was placed in a dish of approximately 300 cercariae on April 22, 1941, later developed 7 lesions.

There does not appear to be an evident dermatitis problem in the San Diego area. Nevertheless, the author has on three occasions developed a number of typical lesions after collecting trips for the snail hosts. Questioning of collectors near the rocky shores and of several bay and beach swimmers revealed that they had had some experience with lesions that might have been of schistosome cercarial origin. Subsequent studies should indicate whether an actual dermatitis problem exists.

Despite the wide distribution of dermatitis-producing schistosome cercariae in the United States, Canada, and other parts of the world, dermatitis-producing

schistosome larvae have not previously been described from marine gastropods. Indeed, the knowledge of marine furcocercous cercariae is very scanty. Nevertheless, the occasional records of mild to severe dermatitis of undetermined etiology contracted by waders and swimmers in marine and brackish waters may be due in part to schistosome cercariae. The evidence that schistosome larvae may be responsible for at least some of the cases of skin irritation contracted in marine waters is strengthened by the present paper recording the presence of known "water itch" cercariae from marine snails.

Although "water itch" has been recognized for more than 50 years, it attracted little attention until relatively recent years, when its relation to schistosome cercariae in snails was widely publicized. According to Cort, et al (1940) it is undoubtedly becoming more common, probably not because of increased snail infection, but because the number of people using the beaches has greatly increased. Certainly if bay and beach populations increase and new recreation areas are developed, as is most obviously true in Southern California, we can expect water itch problems in the areas where infected snails are present. With the increased human activity along the coast lines for recreational and other reasons, there is no question but that people in contact with marine waters will be exposed to *C. littorinalinae* or other dermatitis-producing schistosome cercariae which may be present. The author has already verified the presence of bird type schistosome cercariae in *Cerithidea californica* and *Nassa obsoleta* in California and Connecticut respectively, but has not yet had opportunity to make a detailed study of the cercarial species present or whether they will or will not produce experimental dermatitis in man. Beaches and bays containing large numbers of the host snails are of potential danger even if dermatitis outbreaks are not known to occur. Although "water itch" is not usually serious it is important because its annoyance is an economic detriment in any resort area or area where humans have contact with the water containing the infected snails. The genus *Littorina* is widespread on the Atlantic and Pacific Coasts and also occurs on Asiatic and European shores as well. Thousands of snails may occur at any given place and if there is a high incidence of infection, numerous cercariae may be shed into the waters of bays or the open ocean to reach humans or other animals in contact with the water. One can expect reports of marine "water itch" attributed to schistosome larvae.

SUMMARY

A dermatitis-producing schistosome cercaria, *Cercaria littorinalinae* n. sp., is described from *Littorina planaxis* Philippi, a marine, operculate gastropod which is common along the rocky shores and islands of the California coast. Experimental production of dermatitis is reported from human and animal volunteers.

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THE EFFECT OF THIOURACIL AND THYROID EXTRACT ON THE NATURAL RESISTANCE OF MICE TO *HYMENOLEPIS* INFECTION*

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White mice about 2.5 months old appear to be the most susceptible to an initial experimental infection with *Hymenolepis nana* var. *fraterna,* whereas those past 5 months of age demonstrate the greatest resistance (Shorb, 1933; Hunninen, 1935; Larsh, 1944.) When Holtman (1946) showed that an altered metabolic rate in mice affected their natural resistance to a certain virus, it suggested a possible explanation for the above difference in response to *Hymenolepis* as the metabolic rate differs greatly for mice of these separate age groups. The present experiments were designed, therefore, to test the effect on natural resistance to *Hymenolepis* of depressing the normal high metabolic rate of young mice with thiouracil injections, and elevating the normal low rate of old mice with thyroid extract injections; these results were reported earlier in abstract form (Larsh, 1947a). As far as known, this approach differs from that used in related studies, as in these cases the normal rate of young animals alone was depressed or elevated to determine the effect on natural resistance (Todd, 1948, 1949; Wheeler et al, 1948; Whitlock, 1949).

MATERIALS AND METHODS

The mice of the present study were raised free from *Hymenolepis* on a diet of Purina dog checkers and water. This diet was continued throughout the various experiments. Records of daily food intake (computed by a previously described method, Larsh, 1947b) and weight change during the experiments were necessary as indications of thyroid gland activity of the various groups of mice. These findings, therefore, are given brief discussion in the text. The daily doses of the thiouracil and the thyroid extract (both in powdered form) were suspended in 0.2 cc. of water and forced down the esophagus through a blunted 18 gauge needle attached to a 1.0 cc. tuberculin syringe. The doses of these substances used were based on previous work in which the metabolism of the mice was altered considerably (Holtman, 1946). Although no attempts were made to measure directly the effects on metabolism, there were no visible indications that the amounts of thiouracil and thyroid extract used were toxic. After daily treatment with one of these products for three weeks, these mice and their controls were given alternately a test dose of *Hymenolepis* eggs. The number of cysticercoids observed 93 hours later was used as the criterion of resistance. The methods of isolating and standardizing *Hymenolepis* eggs for infections, of infecting the animals, and of making counts of the 93-hour cysticercoids can be referred to elsewhere (Larsh, 1942).

Student's "t" test was the instrument used to determine the statistical significance of the observed differences in numbers of parasites recovered from the experimental

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* The Thiouracil was supplied by the American Cyanamid Company through the courtesy of Dr. Sterling Brackett.

and control groups. A probability of 5 or less ($P, < .05$), indicating that such a difference could have occurred by chance in not more than 5 of 100 similar experiments, was considered to be significant. In the text, the probability is given in parenthesis in form similar to the above example in instances where it seemed desirable to give some indication of the degree of significance.

EXPERIMENTAL RESULTS AND DISCUSSION

The Effect of Thiouracil on Natural Resistance of Young Mice.—Three experiments were performed. In the first, female mice 1.5 months old were used, and in the second and third experiments equal numbers of both sexes were selected at the age of 1.75 months. In each experiment, the young mice were divided equally into treated and control groups.

In experiment one, the mice of the treated group received daily for three weeks one mg. of thiouracil which was forced down the esophagus as explained above. During this period the controls were given the same treatment with water alone. On the day following the last treatment, all of the mice were infected with 1000 *Hymenolepis* eggs. Table 1 shows the average number and percentage development of cysticercoids as well as data on the average daily food intake and weight change of the mice during the experiment.

The mice given thiouracil (A) had an average of 19.9 cysticercoids, compared with an average of 20.7 cysticercoids for the controls (B). The slight difference in numbers recovered from the two groups is not significant statistically. It is interesting to note that during the three weeks of treatment the mice given thiouracil consumed on the average somewhat more food daily than the controls (5.1 g. and 4.6 g.) while gaining much less weight (5.4 g. and 7.2 g.).

Experiments two and three were similar in design to experiment one, except that the daily thiouracil dosage was increased from one mg. in the first experiment to 2.1 mg. The mice were 1.75 months old at the start of the experiment and received a test dose of *Hymenolepis* eggs of 1400 (experiment two) or 1200 eggs (experiment three). These results are likewise summarized in Table 1.

TABLE 1.—*Showing the number and percentage development of cysticercoids in young mice (A) given thiouracil daily for three weeks before infection and in controls (B)*

Exp. no.	No. mice	Age at infection (months)	Daily dose of thiouracil (mg.)	Infecting egg dose per mouse	Av. daily food intake during exper. (grams)	Av. wght. during exper. (grams)	93-hour cysticercoids		
							Average number	Range in number	Percentage development
A. Young mice given thiouracil									
1	9	2.25	1.0	1000	5.1	gain-5.4	19.9	1–48	1.99
2	8	2.50	2.1	1400	5.4	gain-0.5	44.9	27–74	3.21
3	6	2.50	2.1	1200	5.4	loss-0.2	56.8	20–85	4.73
B. Young mice not given thiouracil									
1	9	2.25	0.0	1000	4.6	gain-7.2	20.7	12–40	2.07
2	8	2.50	0.0	1400	4.9	gain-3.1	46.5	28–61	3.32
3	6	2.50	0.0	1200	5.5	gain-1.5	46.7	18–70	3.89

As for experiment one, the difference in the number of cysticercoids observed in the treated mice of experiments two and three and the number observed in controls is not significant (44.9, 56.8 and 46.5, 46.7, respectively). Thus for the doses

employed in these three experiments, thiouracil did not alter the natural resistance as measured by this criterion. Direct comparisons can not be made with other studies listed in the introduction because of differences in experimental details, such as host-parasite combination used, age of animals, size and schedule of thiouracil doses, etc. However, it is interesting to mention that Holtman (1946) demonstrated a lowered natural resistance to poliomyelitis virus in young mice given thiouracil. He suggested that the lowered metabolism produced by this substance may have been more favorable for virus propagation but made no mention of the possible ill effects of the product on the normal operation of the defensive mechanisms. Others who have fed thiouracil to chickens have not shown an alteration in natural resistance to certain protozoan and helminth parasites (Wheeler et al., 1948; Todd, 1949). The present findings for mice infected with *Hymenolepis* agree with the latter results. Normal condition of the thyroid gland at birth may be found to be associated with these failures to lower resistance, since Whitlock (1949) has shown under controlled conditions that thiouracil fed to a lamb with embryonal type of thyroid caused a fatal outcome of a natural infection of trichostrongyles.

As shown in Table 1, the daily food consumption for mice of experiments two and three was about the same for the treated and control groups (averages, 5.4 g. and 5.2 g., respectively). However, the weight gain was considerably less for the treated mice (average, 0.15 g., compared with 2.3 g. for the controls). These controls, as expected, did not gain at a rate comparable with that of the controls of experiment one, which, being younger, were in a more active growing stage during the experiment. Analysis of the three experiments shows that the food consumption was somewhat similar for the treated and control groups, but the latter gained considerably more weight. This weight difference was most striking in the last two experiments in which the thiouracil dosage was increased. The interference of thiouracil in normal weight gain has been noted by others in related studies (Todd, 1948).

The Effect of Thyroid Extract on Natural Resistance of Old Mice.—In the three experiments carried out, female mice, 6.0 months, were selected for the first experiment, and equal numbers of both sexes, 10.25 months old, were used in experiments two and three. The mice were divided equally into treated and control groups. In each of the three experiments, a third group of mice, 1.75 months old, was included. These mice, of the most susceptible age at the time of infection, were included to check the viability of the eggs used for infecting. Hence they are referred to as viability controls.

In experiment one, the treated mice received daily for three weeks two mg. of thyroid extract which was forced down the esophagus. The young and old controls received the same treatment with water alone. On the day following the last treatment, all of the mice were infected with 1000 *Hymenolepis* eggs (Table 2).

The old mice given thyroid extract (A) had an average of 16.4 cysticercoids, which is not significantly different from the 11.0 cysticercoids for the old controls (B). These results show, therefore, that the thyroid extract as given did not interfere with natural resistance as measured in this way. The young viability controls (C) harbored 18.2 cysticercoids which is statistically greater than the number

found in the old controls (P, < .05), which supports the previous reports mentioned above that age resistance to *Hymenolepis* is developed by this host.

The daily food consumption (5.5 g.) was the same for the three groups but great variation occurred in weight change during the experiment. As expected, the young mice gained (1.9 g.), but both the treated and control groups of old mice lost considerable weight (8.6 and 6.5 g., respectively). No explanation is offered for the weight loss of the controls, unless it is a normal function of the aging process during this period of life. There is support for this suggestion in the fact that a sudden loss of weight often occurs in the aged (Thewlis, 1941). Since the food consumption was similar for both groups of old mice, the slightly greater weight loss of mice in the treated group probably was due to the effects of this product on thyroid activity with an increase in metabolism. Outward evidence for this was much greater activity of the treated mice.

TABLE 2.—*Showing the number and percentage development of cysticercoids in old mice given thyroid extract daily for 3 weeks before infection (A), and in old controls (B) and young controls (C)*

Exp. no.	No. mice	Age at infection (months)	Daily dose of thyroid extract (mg.)	Infecting egg dose per mouse	Av. daily food intake during exper. (grams)	Av. wght. during exper. (grams)	93-hour cysticercoids		
							Average number	Range in number	Percentage development
A. Old mice given thyroid extract									
1	9	6.75	2.0	1000	5.5	loss-8.6	16.4	8–24	1.64
2	9	11.00	3.3	1400	5.7	loss-2.2	70.0	49–90	5.00
3	6	11.00	3.3	1200	5.5	loss-2.2	80.7	42–107	6.73
B. Old mice not given thyroid extract									
1	9	6.75	0.0	1000	5.5	loss-6.5	11.0	0–22	1.10
2	8	11.00	0.0	1400	4.5	loss-3.9	26.9	8–48	1.92
3	6	11.00	0.0	1200	4.4	loss-3.2	41.7	19–66	3.48
C. Young mice not given thyroid extract (viability controls)									
1	9	2.50	0.0	1000	5.5	gain-1.9	18.2	9–29	1.82
2	8	2.50	0.0	1400	5.8	gain-2.3	46.5	20–60	3.32
3	6	2.50	0.0	1200	5.3	gain-3.1	59.1	38–72	4.92

Experiments two and three were similar to experiment one but the daily thyroid extract dose was increased from two mg. in the first experiment to 3.3 mg. The treated and control groups of old mice were 10.25 months old at the start of the experiments and the young viability controls were 1.75 months old. On the day after the last treatment, all of the mice were infected with 1400 *Hymenolepis* eggs (experiment two) or 1200 eggs (experiment three). The results of these two experiments are likewise summarized in Table 2.

In both experiments two and three, the treated mice (A) harbored significantly greater numbers of cysticercoids than the old controls of group B. In the second experiment, the treated mice had an average of 70.0 cysticercoids and the old controls 26.9 (P, < .01), and in the third experiment these averages were 80.7 and 41.7, respectively (P, < .01.). Therefore, unlike those of experiment one, the treated mice had a breakdown in natural resistance. In experiments two and three, respectively, this was so striking that they harbored significantly greater numbers of cysticercoids than the viability controls which were of the most susceptible age, 70.0 and 46.5 (P, < .01.) and 80.7 and 59.1 (P, < .05). Experiments two and

three differed from experiment one in three respects, the age of the old mice used, the number of *Hymenolepis* eggs given at infection, and the dosage of thyroid extract. It is probable that the smaller thyroid extract dose in experiment one was the most important factor in the failure of those mice to show an alteration in resistance. The old controls of the present experiments had developed age resistance as demonstrated for those of experiment one. This is shown by comparing the number of parasites observed in them with the number in the young viability controls, 26.9 and 46.5 ($P, < .02$) and 41.7 and 59.1 ($P, < .05$), respectively. With the use of substances with greater thyroactive properties, e.g., thyroactive iodocasein used by Todd (1949) and others, it may be possible to produce even greater effects on resistance than shown in these experiments.

These results, showing a breakdown in natural resistance following thyroid extract treatment, can not be compared with those of other workers as no reports were found dealing with the effects of thyroid extract or other thyroactive substances on resistance of old animals. As stated above, the reports found concern the effect of such substances on the natural resistance of young animals. Further studies are needed to shed light on the mechanism of the lowered resistance to *Hymenolepis* produced by thyroid extract in the old mice of experiments two and three. Since hyperthyroidism as a "stress factor" is known to drain the vitamin and other stores of the body (Ershoff, 1948), this effect may prove to be related to the lowered resistance. This appears to be a good lead in as much as previous studies on *Hymenolepis* in mice have shown that a similar lowered resistance produced by alcohol, during a comparable time interval, can be cancelled by supplements of certain vitamins (Larsh, 1947b).

As shown in Table 2, the daily food consumption for mice of experiments two and three was similar for the treated mice and the young viability controls (average, 5.6 g.), but the old controls consumed much less food (average, 4.5 g.). Both groups of old mice lost weight during these experiments (average, 2.2 g. for the treated mice, and 3.6 g. for the controls). In analyzing the three experiments, it is noted that the treated mice consumed about the same amount of food in each experiment, whereas the old controls consumed about one gram less in the last two experiments. This reduction may have been due to the use of much older mice in the latter experiments. Since the treated mice were of the same age, it is clear that their greater food consumption was related to the effects of the thyroid extract. This greater food intake probably accounted in large measure for their failure to lose weight at the same rate as the old controls.

SUMMARY

Experiments are described which tested the effect on natural resistance to *H. nana* var. *fraterna* of depressing the normal high metabolic rate of young mice with thiouracil injections, and elevating the normal low rate of old mice with thyroid extract injections. In both cases, the mice were treated daily for 3 weeks before infection. The number of cysticercoids observed 93 hours after giving a test infection to the treated mice and their controls was used as the criterion of resistance.

Thiouracil in daily doses of 1.0 mg. (experiment one) or 2.1 mg. (experiments two and three), although interfering with normal weight gain of the young mice,

failed to alter the natural resistance as measured by this criterion. Thyroid extract given to the old mice in daily doses of 2.0 mg. (experiment one) failed to alter the natural resistance. However, when the daily dose of thyroid extract was increased to 3.3 mg. (experiments two and three) the mice exhibited a breakdown in natural resistance. This was so striking that the treated mice harbored significantly more cysticercoids than young mice of the most susceptible age, which were included in these tests as a check on the viability of the eggs used for infecting. The old controls of these experiments harbored significantly fewer cysticercoids than these young mice, indicating the development of age resistance as shown by previous workers.

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NEW MITES (ACARINA: LIPONYSSINAE) FROM NORTH AMERICAN BATS

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Among collections of bat ectoparasites from California two species of *Ichoronyssus* have been identified, one new and the other previously unrecorded for North America. In the following descriptions the terminology used, with minor exceptions, is that employed by Fonseca (1948).

Ichoronyssus longisetosus, n. sp.

Female, Fig. 1

Body about 650 to 750 microns long by 430 to 450 microns wide; rather sparsely clothed with setae as illustrated. Legs short and stout; the fourth pair slightly longer than the first pair.

Dorsum: Dorsal plate covering approximately 2/3 width of metapodosoma, undivided and measuring about 612 microns long by 277 microns wide; plate indented postero-laterally, with short, median, terminal extension bluntly rounded; some anterior setae up to 55 microns long, the rest except the posterior pair, less than 35 microns long. Peritreme normally rising to dorsal side over coxa III, ending ventrally at the mid-level of coxa I.

Venter: Lacinia of tritosternum ciliated. Shape of presternum as figured, with surface reticulated to form numerous flattened cells. Sternal plate slightly over twice as wide as long at narrowest points; a pair of indistinct antero-lateral areas formed of closely striated cuticula as illustrated. Metasternal setae of approximately same length as sternal setae. Genito-ventral plate with single pair of relatively long setae; plate widening slightly behind level of coxae IV, with broadly rounded end. Anal plate broadly rounded anteriorly; paired setae smaller than unpaired seta which is set on a small pedicel. Unchitinized ventral area of opisthosome with from 46 to 60 pairs of setae measuring approximately 30 microns long except for about 5 to 8 stouter caudal pairs measuring up to 50 microns long.

The measurements tabulated below are in microns and represent the two extremes and the average of five and, in some cases, six specimens.

	TSB	TSL	SL	SW_1	SW_2	SSL_1	SSL_2	SSL_3	MSL	GSL
Smallest	15×16	76	59	125	158	63	63	58	59	49
Largest	17×18	84	63	131	163	68	65	65	63	54
Average	16×17	82	61	127	161	65	63	61	62	48

Explanation of symbols: TSB, tritosternal base, length by width; TSL, tritosternal lacinia; SL, length of sternal plate at mid-ventral line; SW_1, width of sternal plate at narrowest point; SW_2, width of sternal plate at widest point; SSL_1 to $_3$, length of anterior, middle and posterior setae, respectively, of sternal plate; MSL, length of metasternal setae; GLS, length of genital setae.

Gnathosoma: As illustrated, Fig. 1B, 1C. Palpus with medio-ventral process on first segment. Fixed chela of chelicera with an indistinct apical expansion bearing two recurved fang-like hooklets; movable chela unarmed.

Legs: Setation as in Fig. 1A, 1D. Coxae without ventral spurs or heavy spines; coxa II with distinct antero-dorsal tooth; coxae I to III with two and coxa IV with one ventral setae; anterior seta of II and III and single one of IV pedicellate. All coxae with broadly rounded ridges or lines suggestive of vestigial teeth, those of coxa I, however, appearing more like reticulations.

Male, Fig. 2

Dorsum: Dorsal plate about 550 microns long by 319 microns wide; widest at level of coxa III, tapering to a bluntly rounded caudal extremity; sparsely supplied with setae, most of which

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are about 13 microns long; longer anterior and posterior setae measuring about 40 microns. Peritreme extending to anterior margin of coxa II, bordering lateral margin of body anteriorly, and there appearing either ventral or dorsal in position, depending upon degree of compression of the specimen.

Venter: Presternum reticulated. Sterno-metasterno-genital plate extending from level anterior 1/3 of coxa II to posterior border coxa IV; broadest at level of anterior margin coxa II, narrowed between coxae IV but slightly flared before reaching termination just posterior to coxa IV; setae measuring in microns as follows: SSL_1—55, SSL_2—51, SSL_3—48, MSL—45, genital setae 40; the first three pairs of setae overlapping bases of succeeding pairs; plate not reticulated and showing only two pairs of pores and the male organ. Ventro-anal plate with 17 setae, although preanal number probably varies. Unpaired post-anal seta larger than pair flanking anus. Unarmored opisthosome with approximately 17 pairs of delicate setae averaging about 23 microns long; posterior setae much stouter and up to 30 microns long.

Gnathosoma: Fig. 2B, 2C. Palpus without ventral process on first segment, otherwise essentially as in female. Chelicera with 1 long and 2 short processes, the long one curved into a shallow claw measuring 35 microns long, about twice as long as the short ones.

Legs: Essentially same as in female.

Protonymph, Fig. 3

Body size ranging from 350 to 475 microns long by 220 to 280 microns wide.

Dorsum: With two large shields as figured, indistinctly sclerotized. No small shields visible on specimens examined. Anterior shield with 12 pairs of setae; posterior shield with 4 pairs, the three anterior pairs minute. Thirteen pairs of setae distributed over rest of dorsum.

Venter: Plates indistinctly defined. Sternal plate longer than wide, as illustrated, with three pairs of attenuated setae approximately 47 microns long. Anal plate similar to that of adult female. Peritreme rising dorsally over coxa III and not extending anterior to it.

Gnathosoma: As in the female although the ventral process of the first palpal segment not as well developed in the nymph.

Legs: Essentially as described for female except for lesser size.

Types: Described from 6 females, 2 males, and 5 protonymphs. Holotype female, allotype male and paratype protonymph collected from *Corynorhinus rafinesquii intermedius* H. W. Grinnell, the lump nosed bat, by D. S. Longanecker 7 July, 1946, Calaveras Dam, Alameda County, California; deposited with U. S. National Museum, No. 1901. Paratype collection data same as above except for one female from *Corynorhinus rafinesquii pallescens* Miller, collected by P. H. Krutzsch and K. Dixon, 24 July 1946, Borego Palm Canon, San Diego County, California. Paratypes in the author's collection and also in the collection of E. W. Jameson and R. W. Strandtmann.

Comment. *Ichoronyssus longisetosus* has the typical characters of the genus as redesignated by Fonseca (1948) except that the genito-ventral plate of the female is not pointed caudally. This characteristic is not considered here to be of generic significance since it varies even among the species placed in the genus by Fonseca, i.e., *Ichoronyssus diversipilis* (Vitzthum). The closest relative of *I. longisetosus* seems to be *Ichoronyssus diversipilis* (Vitzthum), which may be distinguished by the fact that the metasternal and genito-ventral setae are much shorter (30 and 20 microns long, respectively); the sternal plate of the latter is almost three times as broad as long, and as emphasized by Vitzthum (1918), there are no coxal spurs. This last feature, if correct, is rather surprising. However, Vitzthum makes a point of it by separating *I. diversipilis* from *Lepronyssus* (as *Liponyssus lobatus* (Klti)) in an accompanying key through the fact that the latter possesses a spur on coxa II; in the description of *I. diversipilis* he states "sämtliche coxae, wie gesagt, ohne Sporne."

Ichonoryssus britannicus (Radford 1941)

1941 *Liponyssus britannicus* Radford, p. 311.

Ichoronyssus britannicus (Radford), another rather close relative of *I. longisetosus*, is here recorded for the first time from North America. The species was originally described from *Nyctalus noctula* Schreb, with no locality data given. Through

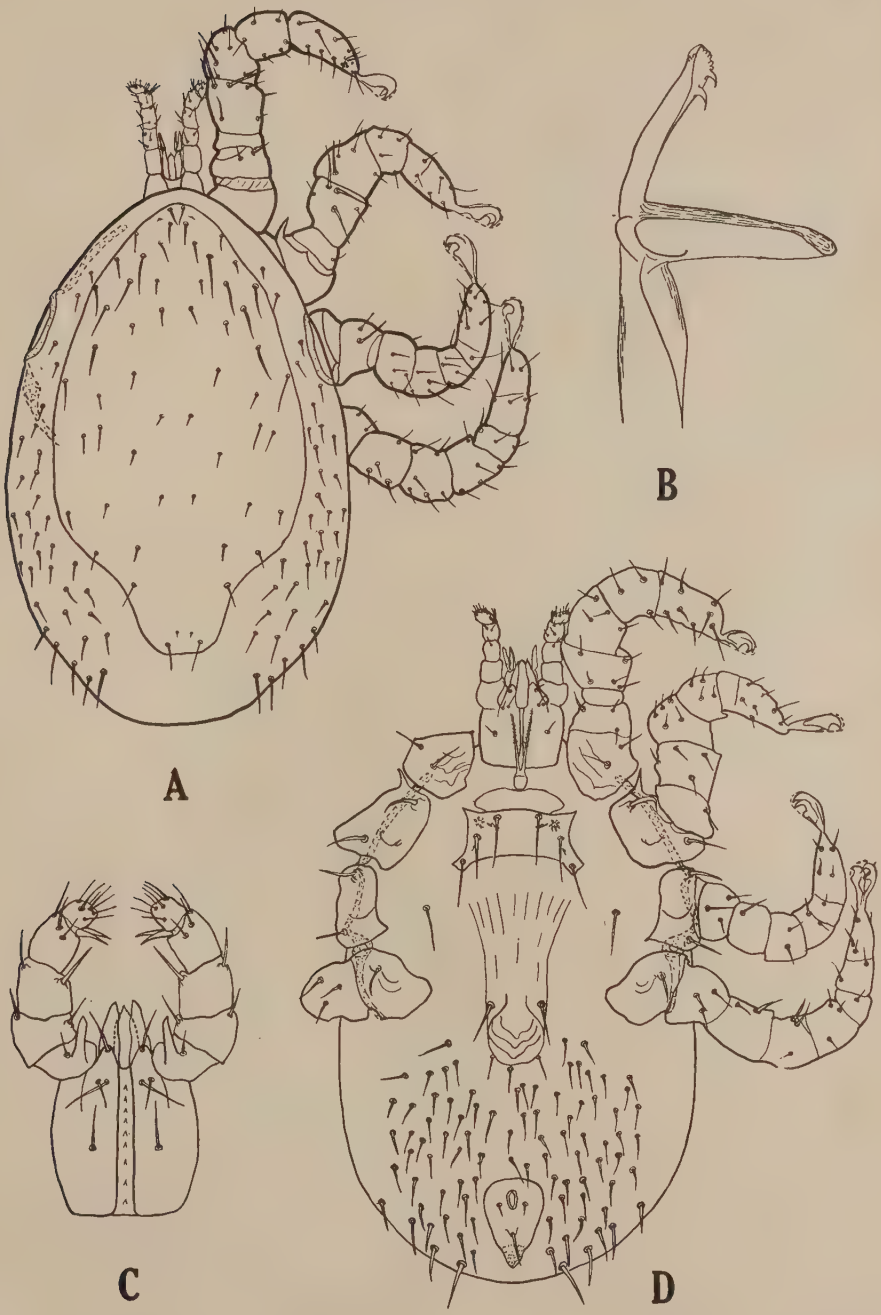

FIG. 1. Female of *Ichoronyssus longisetosus*, n. sp. A, dorsum; B, chelicera; C, ventral view of gnathosoma; D, venter.

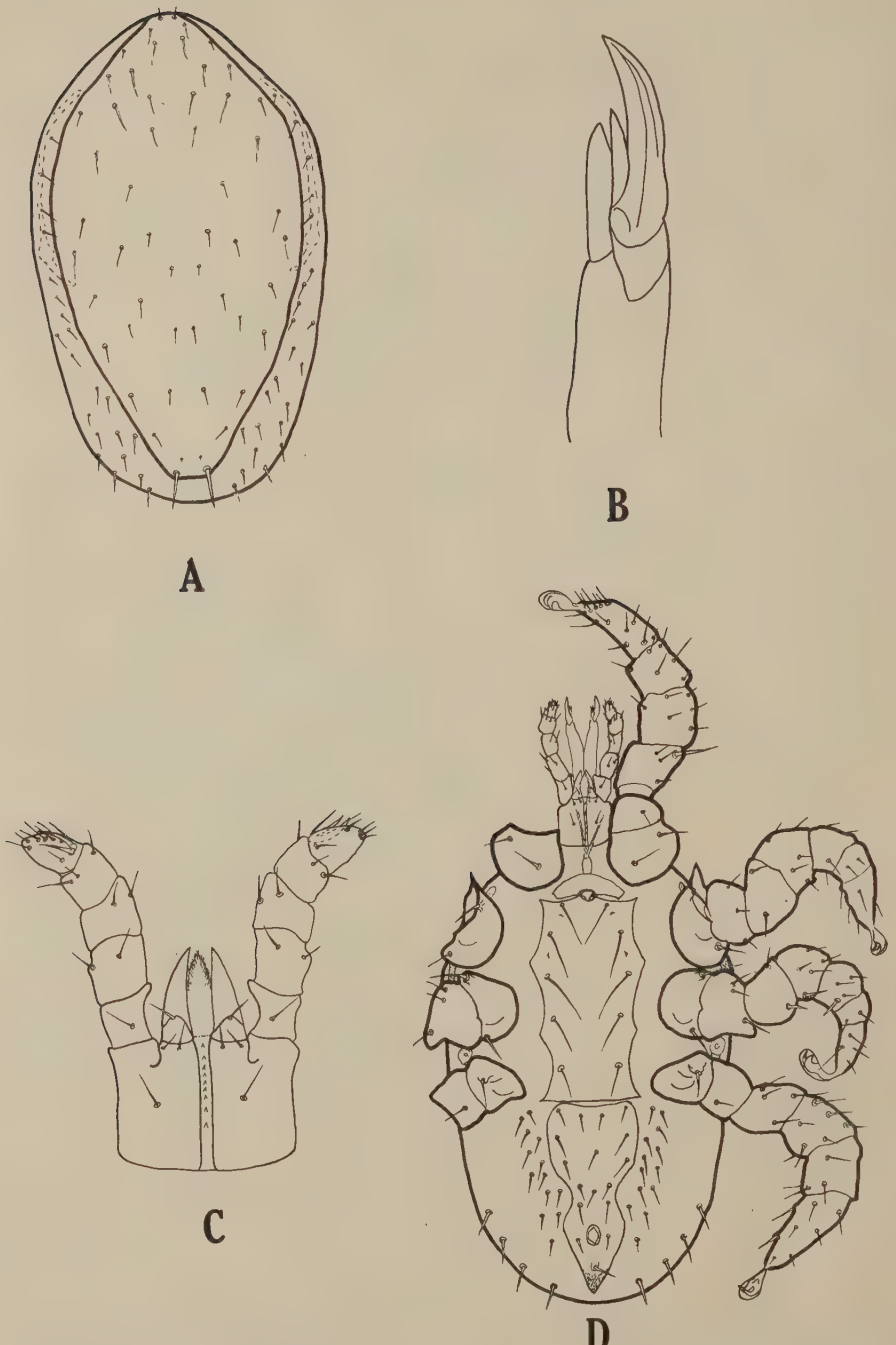

FIG. 2. Male of *Ichoronyssus longisetosus*, n. sp. A, dorsum; B, chelicera; C, ventral view of gnathosoma; D, venter.

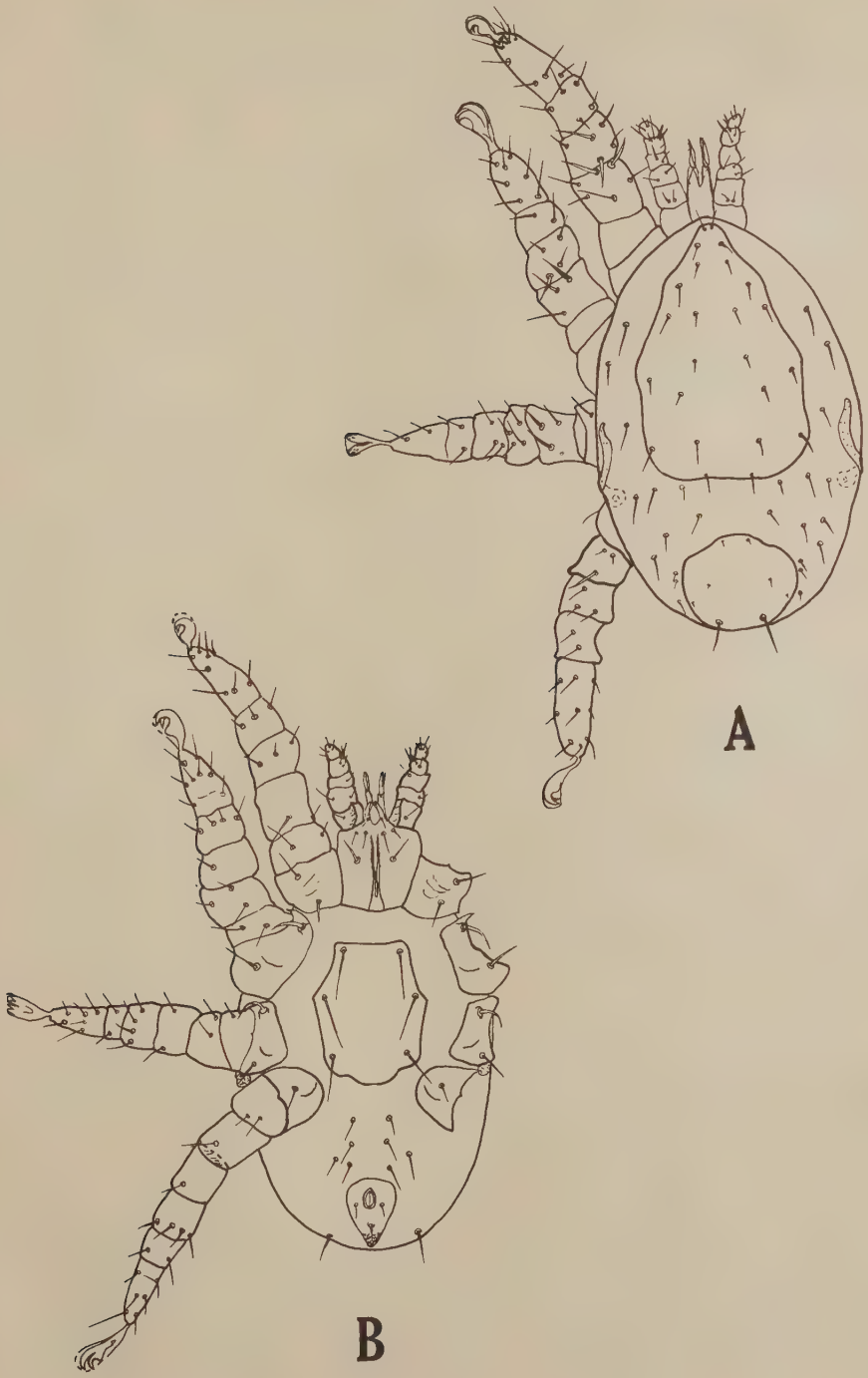

FIG. 3. Protonymph of *Ichoronyssus longisetosus*, n. sp. A, dorsum; B, venter.

the courtesy of Dr. C. D. Radford, the author was able to study paratypes of this species which proved to be identical with the California specimens.

In addition to the original description of *I. britannicus* by Radford it now seems advisable to record the following observations:

Female

Dorsum: Dorsal plate with some anterior setae measuring up to 40 microns, most of the others including posterior pair, not over 16 microns.

Venter: Tritosternum with much shorter base (in both female and male) than indicated in original drawings. Hypostome with a single column of 8 teeth. Presternum distinct (in both female and male) and as described for *I. longisetosus*. Sternal plate with an indistinct pair of antero-lateral areas of closely striated cuticula, similar to those found on *I. longisetosus* but not of the type described for *Hirstesia* Fonseca 1948. Number of setae on the unsclerotized ventral opisthosome varying from 43 to 70 pairs and in addition several minute latero-ventral setae present. Unpaired anal seta set on a small pedicel.

Measurements tabulated below are given in microns and represent the two extremes and the average of 5 to 8 specimens.

	TSB	TSL	SL	SW_1	SW_2	SSL_1	SSL_2	SSL_3	MSL	GSL
Smallest	17.8×16.8	83	49.5	120	149	46	46	45	40	35
Largest	19.8×18.4	92	54	136	172	51	55	55	46	40
Average	19 ×18	86	51.6	126	158	48	51	49	43	37

Gnathosoma: Epistome distinct, elongate triangular with fine lateral ciliation. Immovable arm of chelicera similar to that of *I. longisetosus* in possessing 2 delicate recurved hooklets. Medio-ventral process present on first segment of palpus.

Legs: Half-moon shaped process present on coxa IV as well as II and III.

This species is easily separated from *I. longisetosus* since the female does not have enlarged setae on the caudal extremity, and the first and second holoventral setae of the male do not overlap the bases of succeeding pairs.

Collection data for *I. britannicus* from North America are as follows:

Myotis californicus californicus (Audubon and Bachman), coll. P. Krutzsch and K. Dixon, 20 July 1946, Hubbard's Grove, San Diego County, California; *Myotis thysanodes thysanodes* Miller, coll. P. Krutzsch and K. Dixon, 18 July 1946, 4 miles east of Laguna Junction, San Diego County, California; *Myotis thysanodes thysanodes* Miller, and *Myotis volans interior* Miller, coll. E. W. Jameson Jr., 4 July 1949, near Quincy, Plumas County, California; *Myotis californicus californicus* (Audubon and Bachman), coll. E. W. Jameson Jr., 29 Dec. 1949, Quincy, Plumas County, California.

SUMMARY

Ichoronyssus longisetosus n. sp. is described from the lump nosed bat, *Corynorhinus rafinesquii intermedius* H. W. Grinnell and *C. r. pallescens* Miller, both collected in California. *Ichoronyssus britannicus* (Radford) is reported from three species of *Myotis*, representing the first record of this mite from North America. The original description of this species is amplified.

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INCIDENCE OF *ENTEROBIUS VERMICULARIS* IN PUERTO RICAN CHILDREN, WITH A COMPARISON OF TWO DIAGNOSTIC METHODS[1]

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INTRODUCTION

Stoll and his associates (1947) described surveys in Guam, Marianas Islands, which indicated the extremely low incidence of *Enterobius vermicularis* on that tropical island. Less than one per cent of 634 Guamians of all ages were found to be infected. These investigators concluded that one might expect to find lower rates of infection with this parasite in tropical climates, presenting considerable evidence from the literature, and suggested the need for further data on the incidence of *E. vermicularis* in noninstitutionalized children in tropical communities.

The present work was conducted in Puerto Rico in 1947 mainly for the purpose of comparing the Hall (1937) and Graham (1941) techniques, but enough diagnoses were done to reflect fairly well the incidence in Puerto Rican children.

METHODS

The study was made in the University Hospital and the School of Tropical Medicine in San Juan, on children visiting the pediatric and other clinics. The children, who ranged from 2 to 14 years in age, were subjected to examination without regard to their medical diagnosis. A total of 84 (39 female and 45 male) children were examined. A few had more than one examination so that a total of 97 examinations was made. The examinations always were made between 8 a.m. and 9 a.m., when the patients first appeared at the clinic.

Each patient was examined by two methods. First, an NIH cellophane swab (Hall, 1937) was employed in the usual manner. Immediately after obtaining this swab, the cellulose tape method of Graham (1941) was employed. The gummed side of a loop of tape about 2 in. by $\frac{3}{4}$ in. was applied to the perianal region. The tape then was mounted on a $1\frac{1}{2}$- × 3-in. slide. Both preparations were examined sometime during the day they were taken.

RESULTS

Of the 84 children examined, 3 or 3.6 per cent were positive for *Enterobius* eggs by the NIH swab method, while 10 or 11.9 per cent were positive when the cellulose tape method was used. No positives were found by the NIH swab, which were not detected also by the cellulose tape. However, 7 of those found positive with the cellulose tape were negative on the cellophane swab. The occurrence of eggs of *Trichuris* and *Ascaris* on both the cellulose tape and the NIH swabs was of interest.

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[1] The author wishes to express appreciation to Dr. Jose Oliver-Gonzalez, Dr. P. Morales-Otero, Dr. D. Santiago-Stevenson, and others at the University Hospital and School of Tropical Medicine, San Juan, Puerto Rico, for their assistance and cooperation.

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As shown in Table 1, *Trichuris occurred* in about 17 per cent, and *Ascaris* in about 10 per cent of the cellulose tape samples. As in *Enterobius,* the incidence of *Trichuris* and *Ascaris* as detected by the NIH swab was very much lower. *Trichuris* occurred in only 6 per cent of the swabs, and *Ascaris* in only a single swab (1.2 per cent).

The average numbers of eggs per positive sample also varied widely in the two methods. The positive cellulose tape samples averaged about 116 *Enterobius* eggs per sample, while less than 3 eggs per sample were found in the NIH swabs. For *Trichuris,* the tape averaged about 22 eggs per positive sample, and the NIH swab about 6 eggs. The incidence of *Ascaris* was 19 eggs per positive cellulose tape sample compared to one egg in the single positive found by the NIH swab.

TABLE 1.—*Incidence of 3 intestinal helminths in 84 Puerto Rican children as detected by two diagnostic methods*

	NIH Swab			Cellulose Tape		
	No. Pos.	% Pos.	Ave. No. of Eggs	No. Pos.	% Pos.	Ave. No. of Eggs
Enterobius	3	3.6	2.7	10	11.9	115.7
Trichuris	5	6.0	6.6	14	16.7	22.1
Ascaris	1	1.2	1.0	8	9.5	19.1

It might be noted that, taking into consideration all samples positive by either method, an average of only 0.8 *Enterobius* eggs per sample were detected by the NIH swabs. The average in the cellulose tape samples (115.7) was about 145 times as great. Table 2 summarizes the comparative efficiency of the two methods.

DISCUSSION

Although this survey is very much smaller than that of Stoll (1947), which included 634 persons of all ages, it would seem to provide some additional support to the contention that tropical climates do not favor the transmission of *E. vermicularis.* The only known earlier record of an *Enterobius* survey in Puerto Rico is that of Brady (1941). He found about 20 per cent of 102 children positive by four NIH swabs per child. These children were from an institutionalized group and might be expected to show a somewhat higher incidence than a noninstitutionalized group. The reasons for this low incidence of infection in Puerto Rico are probably those stated by Stoll (1947) and Brady (1941).

No attempt was made to classify the group surveyed according to color or race. Brady (1941) discounts a racial interpretation of the *Enterobius* incidence, and it is presumed that the group which he surveyed was similar to the one here reported.

In this study, there seems to be little doubt that the cellulose tape method of Graham (1941) was superior to the other for accuracy and ease of diagnosis of *Enterobius.* The NIH type swab was used first in each case, followed by the cellulose tape. Even prejudicing the results in this manner, the superiority of the cellulose tape was evident. This superiority already has been demonstrated by Mazzotti and Osorio (1945), Beaver (1949) and others.

The presence of ova other than *Enterobius* on the tape was rather surprising, but similar observations also have been reported by Mazzotti and Osorio (1945), Stoll, *et al.* (1947), and Beaver (1949). Stoll reported presence of hookworm in

TABLE 2.—*Comparative efficiency of individual cellulose tape and NIH swab samples in detecting presence of eggs of* Enterobius Trichuris, *and* Ascaris

Case No.	*Enterobius*		*Trichuris*		*Ascaris*	
	No. of eggs Cellulose tape	No. of eggs NIH swab	No. of eggs Cellulose tape	No. of eggs NIH swab	No. of eggs Cellulose tape	No. of eggs NIH swab
1	33	1	..	..	..	..
3	2	0	..	..	..	..
5	771	2	..	..	..	..
6	..	..	1	0	..	..
11	92	0	..	..	..	..
13	13	0	1	0	..	..
22	24	0	2	0	..	..
27	..	..	3	0	..	..
32	93	0	..	..	..	..
37	..	..	270	6	..	..
38	..	..	3	0	..	..
39	..	..	6	9	..	..
41	..	..	28	1	104	1
52	..	..	1	0	..	..
55	..	..	6	0	..	..
56	..	..	..	..	1	0
60	..	..	..	..	19	0
61	..	..	2	0	..	..
67	..	..	5	0	9	0
79	..	..	2	0	17	0
80	2	0	..	..	..	..
85	..	..	2	2	1	0
86	..	..	1	1	3	0
87	..	..	20	21	..	..
88	..	..	..	..	16	0
91	2	0	..	..	..	..
92	..	..	..	..	2	0
95	125	5	..	..	..	..
Total eggs	1157	8	353	40	172	1
Total Positives	10	3	16	6	9	1
Average no. of eggs per positive sample	115.7	2.7	22.1	6.6	19.1	1
Average no. of eggs per sample positive by either method	115.7	0.8	22.1	2.5	19.1	0.1

8 per cent, *Trichuris* in 11 per cent, and *Ascaris* in 6 per cent of his samples. The present study revealed no hookworm infections, but *Trichuris* was present in about 17 per cent and *Ascaris* in about 10 per cent of the cellulose tape samples.

This occurrence of *Trichuris* and *Ascaris* ova in the cellulose tape samples does not necessarily justify use of this method for routine diagnosis of these infections. In the group surveyed these two parasites infect a fairly high proportion and are usually of a high intensity in the infected individuals. The data on detection of *Trichuris* and *Ascaris* ova are included principally for their value comparing the two diagnostic methods.

SUMMARY

A total of 84 children, 2 to 14 years of age, was examined for *Enterobius* infection in the outpatient clinics of the University Hospital in San Juan, Puerto Rico. Each child was subjected to two diagnostic methods: the NIH cellophane swab, followed by the Graham cellulose tape method. An incidence of 3.6 per cent was detected with the NIH swab, while 11.9 per cent was positive when the cellulose tape was used. The average number of eggs per positive NIH swab was 2.7 as contrasted to an average of about 116 eggs per positive cellulose tape. These results again demonstrate the reported superiority of the cellulose tape method of diagnosis, and provided some additional support to the contention that tropical conditions do not favor transmission of *Enterobius*.

Eggs of *Trichuris* and *Ascaris* also were detected in the samples. An average of 7 *Trichuris* eggs was found in 6 per cent of the NIH swabs, while an average of 22 was detected in 16.7 per cent of the cellulose tape samples. A single *Ascaris* egg was detected in one of the NIH swabs (1.2 per cent) while an average of 19 appeared in 9.5 per cent of the cellulose tape preparations.

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ENDOLIMAX CLEVELANDI, N. SP., FROM TURTLES

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INTRODUCTION

Although representatives of the genus *Endolimax* have been reported for a considerable number of vertebrate and invertebrate hosts (Wenrich, 1935), there are few records of such representatives in reptiles. The amoeba from the European lizard, *Lacerta agilis,* first noted by Hartmann and Prowazek (1907) (named *Amoeba lacertae* by Hartmann in a footnote) and studied more fully by Nägler (1909), and also the one from *Lacerta muralis* (captured near Naples) studied by Dobell (1914), obviously do not belong in the genus *Endolimax.* Their nuclear division stages are different and their mature uninucleate cysts resemble those of certain small free-living amoebae. Hartmann (1914) referred to Dobell's species as *Amoeba (Vahlkampfia) dobelli.* The *"Endolimax reynoldsi"* described by McFall (1926) from the American swift, *Sceloporus undulatus,* with its mature uninucleate cyst, resembles much more the species described by Nägler and Dobell than it does *Endolimax,* and therefore probably does not belong to this genus.

Sanders and Cleveland (1930), in their paper on *Entamoeba terrapinae,* mention finding a small amoeba "similar to *Endolimax nana*" (p. 271) in *Chrysemys picta.* Apparently no cysts were seen. This is the only reference to an *Endolimax*-like amoeba in turtles that we have found. The form seen by these authors is probably the same as that described below.

MATERIALS AND METHODS

The material on which our study is primarily based was obtained from a "Mobile turtle," *Pseudemys floridana mobilensis,* which died in the departmental vivarium in 1943. The rectal contents contained *Octomastix parvus, Trimitus* sp., *Entamoeba* sp., and *Endolimax* sp. Rectal material was placed in cultures and all these Protozoa grew for a time, but the amoebae, while persisting longer than *Octomastix,* did not last as long as *Trimitus.* The *Endolimax* encysted and our cyst material was provided by slides made from cultures. These cultures are mentioned by Wenrich (1947, p. 67). Trophic stages of a similar amoeba were found in fecal material obtained from an unidentified "terrapin" at the Philadelphia Zoological Garden by Q. M. Geiman in 1933.

Description

Trophic stages. The amoeba with which we are concerned, like others in the genus, is quite small. The trophic stages, when rounded or oval, measure 4.7 to 14 μ in diameter with the majority between 5.5 and 8 μ. As a rule, there is slight differentiation into ectoplasm and endoplasm, when the animals are rounded (Figs. 2, 3, 6, 9, 10). However, when pseudopodia are evident, there may be a definite boundary between the finely granular or homogeneous ectoplasm and the coarse endoplasm (Figs. 1, 4, 7, 8). The latter may include clear vacuoles (Figs. 6, 7, 8), or vacuoles containing bacteria, cysts of *Octomastix* or other objects (Fig. 10).

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The single spherical or oval nucleus measures about 1.5 to 3 μ in diameter. Although nuclear size is, in general, proportional to cell size, there may be exceptions. The nucleus is vesicular with a relatively large endosome. In some cases the membrane is faintly stained and difficult to see (Figs. 2, 4, 10), while for other examples, sometimes in the same field of the microscope, the membrane is more definite with a hypothecal layer of chromatin (Figs. 1, 3, 5, 8, 9). The endosome is usually central, conforming in shape to that of the membrane, but occasionally it is eccentric (Fig. 9) or even in contact with the nuclear membrane. It usually has a roughened contour, often with projections which grade into fine radiating strands extending to the periphery (Figs. 1, 2, 3, etc.). As with the membrane, there may be variations in the stainability of the endosome. As a rule it appears to be compact and uniformly stained, but occasionally the central area may be lightly stained (Figs. 4, 6). With haemalum staining there frequently is a deeply-stained granule (centriole?) in the more lightly-stained central area (Fig. 6).

Cysts. As previously indicated, the cysts were found in cultures and the description is necessarily based upon slides made from that source.

The precystic amoebae are usually rounded and smaller than the corresponding trophic stages (Figs. 11–13). The cytoplasm is commonly homogeneous and more deeply stained, both conditions being correlated with the lack of the miscellaneous contents seen in the trophic stages. The nuclear size is proportional to the cell size although often somewhat greater than in trophic animals of the same size. The nuclear membrane is definite and apparently thick or provided with a hypothecal layer of chromatin. The endosome may exhibit various conditions, sometimes suggesting preparation for division, and the radial extensions are less evident. Binucleate precysts have been seen (Fig. 14).

The cysts are typically *Endolimax*-like in appearance. They may be round but more often they are oval or elliptical, but may have other shapes (Figs. 16 to 24). The averaged diameters range from 4.5 to 10 μ. The thin cyst wall seems to consist of a single layer, and the cytoplasm is finely granular. The mature cyst contains four nuclei which resemble those of the trophic stages in appearance but are smaller. No supernucleate cysts were seen. Contrary to the general belief that cysts are always free from foreign bodies, we have several times found a cyst of *Endolimax* containing a cyst of *Octomastix parvus* (Fig. 20).

Many of the cysts in this culture material showed signs of degeneration, including irregularities in contour, coarsely reticular or vacuolated cytoplasm and various nuclear changes. The latter included enlargement (Figs. 22–24) and scattering of endosomic materials into granules of various sizes, often with connecting strands (Fig. 24). Sometimes these hypertrophied nuclei fused into large vesicles of various shapes, which in some instances occupied most of the space within the cyst (Fig. 24). Although extended search was made for them, no nuclear divisions were recognized within the cysts.

DISCUSSION

Although the nuclear structure of *Endolimax nana* may serve to distinguish it from the other genera of amoeba living in man, there are superficial resemblances between this nucleus and those of certain other amoebae. Irregularities in the shape and structure of the endosome of *E. nana,* as insisted upon by Dobell (1943), do occur, but there is some question about their being typical. Most illustrations of *E. nana* in the literature, as well as those of species from other hosts, show a rounded, compact endosome. Hence this condition of the endosome appears to be characteristic, as it is for many free-living amoebae and also for other Protozoa. Furthermore, a hypothecal layer of chromatin on the inner surface of the nuclear membrane is also characteristic of *E. nana* as it is for certain free-living amoebae. Most illustrations of *E. nana* fail to show this hypothecal layer. Stabler (1932) called attention to variations in its appearance correlated with differences in fixing agents, especially the amount of acetic acid in the fixatives. Wenrich (1941) showed in addition that there could be much variation in stainability both of the endosome and the hypothecal layer even with a single fixing agent, as we have indicated above for *E. clevelandi.*

Since the nuclear structure of the trophic stages of *E. nana* shows superficial resemblances to that of some of the free-living and other kinds of small amoebae,

generic allocation on the basis of trophic stages alone could be erroneous. Attention is therefore called to the mature cysts which are characteristic in size, shape and nuclear condition. *Amoeba lacertae* Hartmann (1907), *Amoeba lacertae* Dobell (1914) (= *Amoeba* (*Vahlkampfia*) *dobelli* Hartmann, 1914) and "*Endolimax reynoldsi*" McFall (1926), all from lizards, have uninucleate mature cysts and other characters which indicate that they do not belong in the genus *Endolimax*. Since the small amoeba from turtles here described has cysts very similar to those of *E. nana*, allocation to the genus *Endolimax* seems to be unquestionable. Although *E. clevelandi* resembles *E. nana* very closely, it is unlikely that the latter would live in reptiles. We are dedicating this species to Dr. L. R. Cleveland who, with his associates, first recognized the occurrence of a species of *Endolimax* in a turtle.

SUMMARY

Endolimax clevelandi, n. sp., was found in the rectal contents of a Mobile turtle, *Pseudemys floridana mobilensis*, along with *Entamoeba* sp., *Trimitus* sp., and *Octomastix parvus*. In cultures, *Endolimax*-type cysts were formed.

Rounded trophic stages measured 4.7 to 14 μ in diameter. The usually spherical nucleus was 1.5 to 3.0 μ in diameter. Some nuclei had indistinct boundaries, but others, on the same slide, showed well-defined nuclear membranes bearing a hypothecal layer of chromatic materials. The relatively large and centrally located endosomes were usually solid in appearance, but sometimes displayed a more lightly staining central area which, in haemalum-stained slides, might contain a central granule. Occasionally the endosome was eccentric. It was typically round with ragged edges, with some fine strands extending to the periphery.

Precystic stages resemble the trophic stages but were smaller and lacked inclusions.

Cysts were similar to those of *E. nana* in size and shape, being 4.5 to 10 μ in diameter when rounded, most being oval or elliptical and containing four nuclei in the mature condition. Occasionally a cyst contained a cyst of *Octomastix parvus*. Many of the cysts, which were from cultures, showed signs of degeneration, with hypertrophied nuclei.

Since the nuclear structure of *Endolimax* shows superficial resemblance to certain free-living and other amoebae, it is pointed out that the cyst stage offers better criteria for generic allocation than does the trophic stage.

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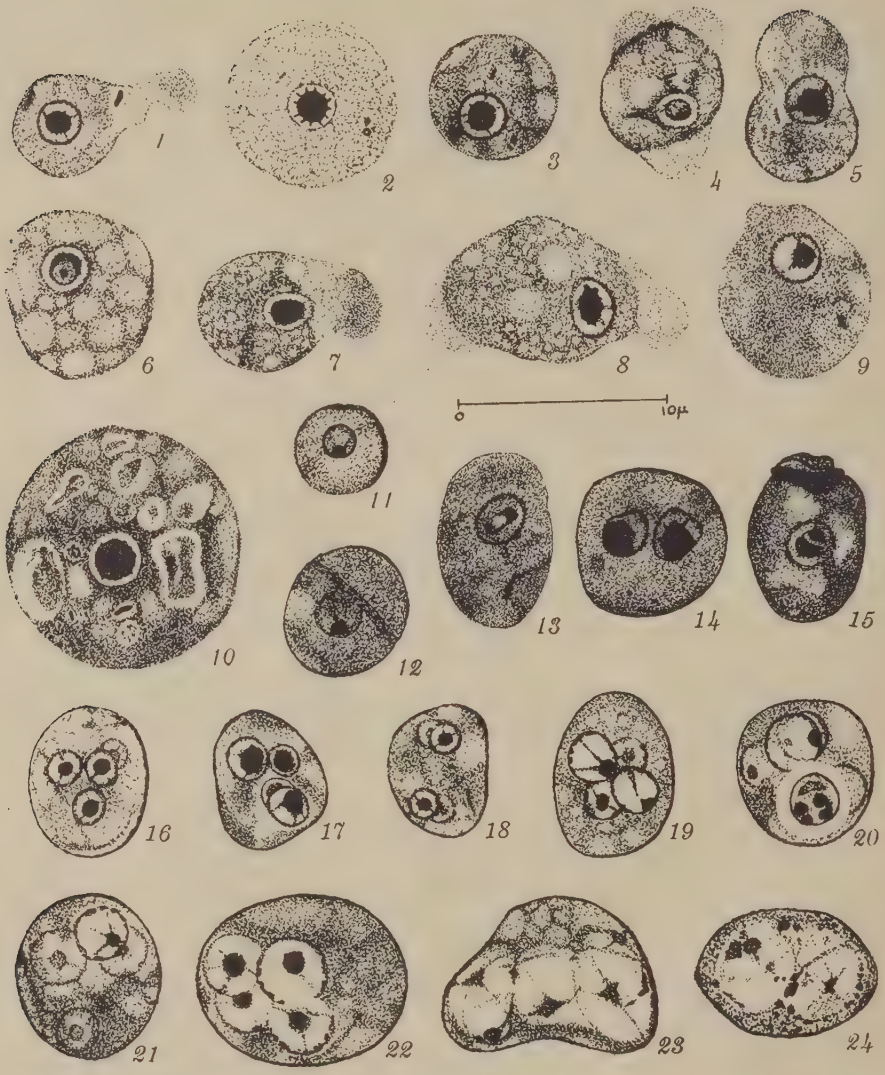

EXPLANATION OF FIGURES

Drawings were made by E. G.-B. at a magnification of × 3000. Unless otherwise indicated, they were made from slides fixed with Schaudinn's fluid plus acetic acid, and stained with Heidenhain's hematoxylin.

FIGS. 1–10. Trophic stages.

FIG. 1. Small typical amoeba.

FIG. 2. Animal with more lightly stained cytoplasm and nuclear membrane.

FIG. 3. Animal with definite hypothecal layer of chromatin material.

FIG. 4. Amoeba with pseudopods; central portion of endosome faint; hypothecal layer indistinct.

FIG. 5. Nuclear membrane more distinct; endosome has few radiating strands.

FIG. 6. Small granule (centriole?) in clear area within the endosome (hemalum).

FIGS. 7 and 8. Amoeba from another host.

FIG. 9. Endosome in nucleus is eccentric.

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FIG. 10. Large specimen showing numerous food bodies.
FIGS. 11–15. Precystic stages.
FIGS. 11 and 12. Endosomes are small and eccentric.
FIG. 13. Endosome is elongated and vacuolated.
FIG. 14. Binucleate condition.
FIG. 15. Unusual nuclear structure; food cup (?) above.
FIGS. 16–19. Normal 4-nucleated cysts.
FIG. 20. Cyst containing cyst of *Octomastix parvus*.
FIGS. 21–24. Cysts showing different stages of nuclear hypertrophy and degeneration.

RESEARCH NOTES

THE COPPERHEAD SNAKE AS A HOST FOR THE CHIGGER MITE *TROMBICULA (EUTROMBICULA) ALFREDDUGÈSI*

Six specimens of the copperhead snake, *Agkistrodon contortrix* (Linnaeus) (vide Klauber, 1948, Copeia, 1948: 1–14), were collected in the Duke University Forest, Durham, North Carolina, during the months of July, August and September, 1949. Four of the six specimens were found to be infested with the chigger mite, *Trombicula (Eutrombicula) alfreddugèsi* (Oudemans, 1910), the common pest chigger in this area. This apparently establishes a new host record for this mite.

Brennan (1945, Tex. Rept. Biol. and Med., 3: 112–121) reported *T. (E.) alfreddugèsi* on the western diamond-back rattle snake, *Crotalus atrox* (Baird and Girard), in Texas, but found none of these mites on copperheads examined. From chigger specimens in the United States National Museum and from his own observations Jenkins (1948, Am. J. Hyg., 48: 22–35) compiled host lists for the three common pest chiggers occurring in the United States. He reported that he was unable to find larvae on any snakes of the family Crotalidae (pit vipers). In a more recent publication he (Jenkins, 1949, Ann. Ent. Soc. Am., 42: 289–318) listed one crotalid, the diamond-back rattlesnake, as a host for *T. (E.) alfreddugèsi.*

During the present study a total of approximately 260 larval trombiculids were recovered from four of the six copperheads examined. The reptiles were identified by J. R. Bailey and all of the mites studied microscopically were identified as *T. (E.) alfreddugèsi* by C. E. Farrell or the author.

Attempts were made to culture some of the larvae from three of the infested snakes. Several larvae from the second specimen were detached mechanically and placed in special plaster-lined vials (Farrell and Wharton, 1948, J. Parasitol., 34: 71) for transformation into nymphs. Three nymphs emerged but all died before metamorphosing into adults. After snake No. 5 had been injected with formalin three of eight chiggers removed were put into transformation vials. All died before metamorphosing into the inactive nymphochrysalis stage. The sixth copperhead was brought to the laboratory alive and placed in a cage over a funnel to collect the larval mites as they became engorged and dropped from their host. Most of those that appeared to be engorged were placed in the special vials for culturing. Many of the larvae transformed into nymphs and later into adults. Eggs of *Aëdes aegypti* were added to the cultures as food for these free living stages. The adults were maintained in the culture and subsequently larvae were produced, thus completing the life cycle. That larvae collected from the copperhead snake were reared through the nymphal and adult stages and first generation unengorged larvae were produced suggests that this reptile is a satisfactory host for *T. (E.) alfreddugèsi.*

This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.—K. E. Hyland, Jr., *Department of Zoology, Duke University, Durham, North Carolina.*

THE SIGNIFICANCE OF FINDING CLONORCHIASIS IN PERSONS IN THE UNITED STATES

In a recent article Edelman and Spingarn (J.A.M.A., 140: 1147–1150) reported the diagnosis of clonorchiasis in 4 patients in New York City who had lived in Shanghai, China, for several years. In their text the authors included the statement, "To our knowledge these are the first cases of this disease among members of the white race to be observed in this country." It is further stated, "The discovery of these cases is another example of the global dissemination of regional diseases by the movement of populations as a result of the last war."

Because my colleagues and I have noted a number of cases of clonorchiasis in the Mayo Clinic, it would appear pertinent to call attention to these cases and comment on the significance of *Clonorchis sinensis* in the United States.

We have observed occasional cases of clonorchiasis over the past thirty years in returned missionaries and other persons who have lived in the Orient in areas in which *Clonorchis sinensis* is endemic and epidemic. There has always been a considerable traffic between China and the United States but there is nothing in the history of the 4 patients reported by Edelman and Spingarn which indicates that the war was responsible for dissemination of clonorchiasis in these instances. The first patient was from Manchuria and had lived in China for fifteen years. The second and third went to China two years before United States was involved in the war. The fourth was born in China four years ago and came to America after the war.

Clonorchis infections have been noted in persons in the United States without reference to

the movement of populations as a result of the last war. During the past ten years my colleagues and I have seen 9 persons in whom we have diagnosed the presence of *Clonorchis sinensis.* Of these people 5 are white, one having a national origin from Russia, two from Lithuania and two from the United States. In addition we have seen 1 California-born Chinese with the disease, 2 persons born in China and 1 in Korea. All of these people lived for varying periods of time in China and in Korea, where the disease is common.

In passing, one should call attention to the fact that the certain diagnosis of *Clonorchis* infection from the eggs alone is not a simple determination. As a matter of fact it is likely that a number of errors occur in reference to this diagnosis. There are several other small flukes, parasitic in man, which have eggs so nearly like those of *Clonorchis* that the differentiation is difficult. Indeed, under certain circumstances it may even be impossible. Since it is usually impossible to obtain the worms of *Clonorchis* from the patient, one is sometimes left in doubt as to the exact identification of the genus and species.

The significant question about *Clonorchis* with reference to patients who live in the United States is whether there is a suitable snail host in which the infection may be propagated. Of course, infection depends not only on the snail host but on suitable species of fishes as well. Moreover, there must be the habit of eating these fishes raw. Fortunately, not many people in the United States have the habit of eating raw fish but there are groups of people who regularly do so. This is manifested by the rather common occurrence of *Diphyllobothrium latum* in certain groups of people in the United States, in particular Jewish housewives and people of Finnish and Swedish origin.

In the past, public health officials have rested rather content in thinking that there were no suitable snail hosts in the United States for the propagation of certain parasitic diseases caused by trematodes. The finding of suitable hosts for the transmission of *Schistosoma mansoni* and *Schistosoma japonicum* in the United States has therefore alarmed public health officials. Up to the present time no snails in this country have been demonstrated to be capable of transmitting *Clonorchis;* however *Bulimus tentaculatus* is present in the Great Lakes region. Until this problem is thoroughly investigated the security which seemed justified in the past is no longer certain. However, unless the habits of Americans change radically with respect to consuming raw fish, the threat to the public from *Clonorchis* will remain negligible.—THOMAS B. MAGATH, M.D., *Division of Clinical Laboratories, Mayo Clinic, Rochester, Minnesota.*

FEEDING HABITS OF *TRIGONOTYLUS RUFICORNIS* GEOFF

The literature contains numerous references to phytophagous insects which occasionally attack man and provoke irritations by the injection of salivary juices in their successful or unsuccessful attempts to secure blood. This note records the reactions of a human host to the attack of *Trigonotylus ruficornis* Geoff., a normally phytophagous heteropteran common in the New York City area. On August 8, 1949 at 9:30 P.M. several individuals of this species were observed crawling on the forearm of an adult male subject who was working in the light field of a 100 watt desk lamp. The air temperature of the light field was 86° F. Two of the insects were permitted to remain on the arm and after a period of exploration each inserted its piercing apparatus at approximately the same moment. A summary of the reaction follows.

Time in minutes	Phenomenon
0	Piercing structures inserted.
0.16	Sharp burning sensation of 10 seconds duration followed by a milder sensation of burning which persisted throughout the remainder of the episode.
1.0	Insects removed.
2.0	First indication of wheals.
7.0	Wheals reached maximum diameter of 4 mm.
22.0	Regression of wheals initiated.
62.0	Wheals disappeared, burning sensation terminated.

Blood cells could not be detected in the stomachs of the insects although there is the possibility that clear tissue fluid could have been withdrawn. This note indicates that under certain circumstances these insects may be responsible for some of the annoyance to humans usually attributed to known bloodsucking forms.—GEORGE S. TULLOCH, *Department of Biology, Brooklyn College, Brooklyn, N. Y.*

PRELIMINARY NOTE ON THE LIFE CYCLE OF THE ACANTHOCEPHALAN, *POLYMORPHUS KENTI* VAN CLEAVE, 1947.

During the winter of 1949, the intestinal tracts of five Western gulls *(Larus occidentalis)* collected at Newport, Oregon, were examined and 35 acanthocephalans were found in one gull. The parasite was identified by Dr. H. J. Van Cleave as *Polymorphus kenti* Van Cleave, 1947. An examination of three Western gulls and one glaucous gull (*Larus glaucescens*) collected at Coos Bay, Oregon, during the summer of 1949, yielded 18 *P. kenti* from the intestine of the glaucous gull; the Western gulls were negative.

This is the first report of the species since its description by Van Cleave (Trans. Amer. Micr. Soc. 66: 302–313; 1947). He reported three specimens from the intestine of *Larus argentatus smithsonianus* collected at Kent Island, New Brunswick, Canada. Finding this acanthocephalan in *L. occidentalis* and *L. glaucescens* in Oregon represents new host and distribution records for *P. kenti.*

During a routine examination of the sand crab, *Emerita analoga* at Coos Bay, Oregon, organisms, which were identified by Dr. Van Cleave as juvenile acanthocephalans, were found free in the haemocoele in the region of the mid-gut. The most common site was ventral to the mid-gut near the digestive gland. A number of these juvenile forms were fed by a pipette to two laboratory rats on July 9, 1949. The rats were killed and examined Aug. 2, 1949, 25 days after feeding, and one immature female 10 mm. long of *P. kenti* was found embedded in the intestinal mucosa of one of the rats. Three rats were fed several juvenile acanthocephalans on Aug. 6, 1949, and examined Sept. 27, 1949, 53 days after feeding. Two rats were infected, each with one *P. kenti,* and the other contained 2 parasites. All four specimens were immature females, the lengths of which were 11, 11, 12, and 15 mm. Van Cleave included the body length for only one specimen which was an immature female of 10 mm. in length.

A survey was made of 109 *Emerita analoga* collected at Coos Bay, Oregon, Aug. 6, 1949. Of this total 86 were mature animals and 23 were immature. Of the adult *E. analoga,* 82 were found to be infected, a 95% incidence. The range of infection was 1–17 with a median of 3. Of the 23 immature *E. analoga,* 12 were infected, of which only one contained 2; each of the remainder were infected with only one juvenile parasite. None of the earlier stages of the life cycle were observed in *E. analoga.*

The habitat of the intermediate host is in the intertidal sand. The probable life cycle of *P. kenti* is: Eggs are passed in the feces of the gull; *E. analoga* are filter feeders and could pick up the eggs; development of the acanthor, acanthella, and the juvenile takes place in the sand crab. The gull could become infected by eating *E. analoga.* However, no remains of *E. analoga* have been observed in the nine gulls examined post mortem.

The author would like to thank Dr. Van Cleave for the identification of the material as stated. The work was conducted at the Oregon Institute of Marine Biology in the laboratory of Dr. Ivan Pratt.—Donald J. Reish, Hancock Fellow, *Allan Hancock Foundation, University of Southern California, Los Angeles, California.*

THE NEMERTEAN, *CEREBRATULUS LACTEUS,* AS AN INTERMEDIATE HOST FOR CESTODE LARVAE

On August 24, 1949, at the Duke University Marine Laboratory, Beaufort, N. C., approximately 120 tetraphyllidean plerocercoid larvae were found when a single specimen of *Cerebratulus lacteus* (Leidy) fragmented. It is believed that this is the first known record of *Cerebratulus* as a host for larval helminths.

There were no signs of cysts around the worms and the larvae were apparently free in the tissue spaces of their host. A few were adhering to the outer walls of the digestive tract, but exact location of the parasites could not be determined because of the condition of the host.

Positive identification to genus only is possible at present, due to the fact that scolex anatomy is the sole basis for study and comparison. Although scolex characters are important in the classification of the Tetraphyllidea, definite placement of a cestode should be determined by characteristics of the proglottids as well. The cestodes belong to the genus *Echeneibothrium* Van Beneden 1850. There is a very close resemblance of the scolices of these larvae to those of the adult *E. maculatum* described by Woodland in 1927 (Proc. Zool. Soc. London 1927: 518–548). However, according to various authors (Southwell, T., The Fauna of British India: Cestoda, I. London 1930) the myzorhynchus characterizing certain species of *Echeneibothrium* may be present in young forms and abortive in the older worms. Therefore, one cannot make specific determination with any degree of assurance until all stages of the worms are available. This report is important only because of the unusual host record and its implication of probable host relationships in the Beaufort area.—Wanda Sanborn Hunter, *Department of Zoology, Duke University, Durham, North Carolina.*

A PRELIMINARY HOST-ECTOPARASITE REGISTER FOR SOME SMALL MAMMALS OF FLORIDA

During the course of mammal and ectoparasite studies in 1948 and 1949, chiefly in Hillsborough County, Florida, records of host-ectoparasite associations were maintained. These are shown in the accompanying table. Although the list is obviously far from complete, it is felt that the information may be of some value, especially since in numerous instances it is possible to appraise the status of a given ectoparasite in relation to its host or hosts.

Data are based on roughly the following numbers of mammals: 500 Norway rats, *Rattus norvegicus* (Erxleben); 2,800 roof rats, *Rattus rattus* (Linnaeus) subsps.; 25 house mice, *Mus musculus* Linnaeus subsp.; 900 cotton rats, *Sigmodon hispidus* Say and Ord subsps.; 100 rice rats, *Oryzomys palustris* (Harlan) subsps.; 30 wood rats, *Neotoma floridana floridana* (Ord); 50 cotton mice, *Peromyscus gossypinus palmarius* Bangs; 30 gray squirrels, *Sciurus carolinensis carolinensis* Gmelin; one pocket gopher, *Geomys floridanus austrinus* (Bangs); two cottontail rabbits, *Sylvilagus floridanus floridanus* (Allen); 15 marsh rabbits, *Sylvilagus palustris paludicola* (Miller and Bangs); one raccoon, *Procyon lotor elucus* Bangs; 40 opossums, *Didelphis virginiana pigra* Bangs; and one short-tailed shrew, *Cryptotis parva floridana* (Merriam).

Acknowledgment for the identification of many of the ectoparasites by appropriate experts has been made in other publications dealing with these mammals and arthropods. Full names of the ectoparasites, abbreviated in the table for the sake of compactness, are:

Acarina:

1. *Liponyssus bacoti* (Hirst)
2. *Laelaps nuttalli* (Hirst)
3. *Laelaps* sp.
4. *Laelaps echidninus* (Berlese)
5. *Haemolaelaps glasgowi* (Ewing)
6. *Haemolaelaps* sp.
7. *Androlaelaps setosus* Fox
8. *Cosmolaelaps gurabensis* Fox
9. *Gigantolaelaps* sp.
10. *Radfordia ensifera* (Poppe)
11. *Cheyletus eruditus* (Schrank)
12. *Dermatophagoides scheremetewskyi* Bogdanow
13. *Eutrombicula batatas* (Linnaeus)
14. *Eutrombicula splendens* (Ewing)
15. *Eutrombicula multisetosa* (Ewing)
16. *Eutrombicula* sp.
17. *Listrophorus* sp.
18. *Dermacentor variabilis* (Say)
19. *Ixodes scapularis* Say
20. *Ixodes* sp.
21. *Amblyomma maculatum* Koch
22. *Haemaphysalis leporis-palustris* (Packard)

Anoplura:

23. *Polyplax spinulosa* (Burm.)
24. *Hoplopleura oenomydis* Ferris
25. *Hoplopleura hirsuta* Ferris
26. *Hoplopleura quadridentata* (Neumann)
27. *Neohaematopinus sciurinus* (Mjob.)

Mallophaga:

28. *Geomydoecus scleritus* McG.

Siphonaptera:

29. *Xenopsylla cheopis* (Rothschild)
30. *Pulex irritans* (Linnaeus)
31. *Leptopsylla segnis* (Schonherr)
32. *Ctenocephalides canis* (Curtis)
33. *Ctenocephalides felis* (Bouché)
34. *Echidnophaga gallinacea* (Westwood)

35. *Polygenis gwyni* (C. Fox)
36. *Orchopeas howardii* (Baker)
37. *Cediopsylla simplex* (Baker)

Diptera:
38. *Cuterebra* sp.
39. *Dermatobia*-like sp.

Host-Ectoparasite Records from Florida Mammals

	Norway rat	Roof rat	House mouse	Cotton rat	Rice rat	Wood rat	Cotton mouse	Gray squirrel	Pocket gopher	Cottontail rabbit	Marsh rabbit	Raccoon	Opossum	Short-tailed shrew
L. bacoti	C	C	U	C	C		C				U		U	
L. nuttalli	C	U						R						
Laelaps sp.				R	C									
L. echidninus	C	U												
H. glasgowi		C	U	C	C		C							U
Haemolaelaps sp.									U					
A. setosus		R												
C. gurabensis		R												
Gigantolaelaps sp.					C E									
K. ensifera		R	R											
C. eruditus		R												
D. scheremetewskyi							U E							
E. batatas		U E			U E									
E. splendens				C	C									
E. multisetosa				U E	U E									
Eutrombicula sp.			U					U		U		U	U	
Listrophorus sp.			U	C	C		C							
D. variabilis		U		C	C		C				U	U	U	
I. scapularis											U		C	
Ixodes sp.				U	U		U							
A. maculatum		U		U										
H. leporis-palustris		R								U	C			
P. spinulosa	C	C												
H. oenomydis	U													
H. hirsuta				C										
H. quadridentata					C									
N. sciurinus								U						
G. scleritus									U					
X. cheopis	C	C		R		R							R	U
P. irritans													U	
L. segnis		R												
C. canis				U G										
C. felis		U											U	
E. gallinacea	C	C	U	R				U	U				C	
P. gwyni	R	R		C	C	C	C						U	
O. howardii								C						
C. simplex											C			
Cuterebra		U				U					C			
"Dermatobia" sp.		U E												

C—Common.
R—Rare.
U—Status unknown; recorded irregularly or from only a small series of hosts.
E—Everglades only.
G—Gadsden County only.

The work reported in this note was performed with the support and under the auspices of the International Health Division of The Rockefeller Foundation in co-operation with the Florida State Board of Health.—C. Brooke Worth, *Box 4298, Tampa, Florida.*

DISPERSAL FLIGHT OF *TRIATOMA* IN SOUTHERN ARIZONA

Periodically since the middle 19th century, reduviid bugs have appeared in variable numbers and received unusual public notice ("kissing bug" scare) as a result of painful bites received by man (Howard, 1899, Pop. Sci. Mo. *55,* 31–42). In Arizona, *Triatoma* (*Conorhinus*) has been known for years as a house invader due to its pestiferous habit of feeding on man, especially during the warmer months of the year (Morrill, 1914, Arizona Med. Jour., Jan., 1–12). Wehrle (1939, Bull. Brooklyn Ento. Soc., *34,* 145–154) recorded observations on the dispersal flight of these bloodsucking bugs and their numbers in wood rat dens. Wood (1941, Pan-Pacific Entomologist *17,* 85–94, 115–118) collected an average of 2.88 bugs per house from 451 wood rat

houses throughout southwestern United States, finding a maximum of 85 bugs from one den in southern California. Wood (1943, Am. Jour. Trop. Med. *23,* 315–320) observed nocturnal flights of these insects in the field. The same type of house-invading activities and annoyance to man recorded by Wehrle have been noted by the writer (Wood, 1941, Am. Jour. Hyg. *34,* 1–13).

The variation in numbers of house-invading *Triatoma* during successive seasons is well illustrated by the writer's observations at the Alvarado Mine in west-central Arizona (Wood, 1943). A close inspection of the concrete wall and foundation area of the old mill site at the mine at midnight of July 15, 1949 revealed no *Triatoma.* Apparently, no bugs visited three campers during the night because of the coolness. Thus, there is yearly variation in populations of *Triatoma* with some seasons revealing unusual numbers of individuals. If this great increase in insects occurs near concentrations of human habitations it may receive widespread public notice.

An occurrence of large numbers of *Triatoma rubida uhleri* in dispersal flight was noted by Usinger (1944, Public Health Bull., No. 288) in June of 1930 at Peach Springs, Arizona, when many bugs were collected in the daytime, presumably after a widespread night flight. A similar flight involving *Triatoma longipes* and *Triatoma rubida uhleri* occurred on July 19th or 20th, 1948, near Nogales, Arizona, as reported by Mr. W. J. Cummings. His letter of February 23, 1949, to the writer records the following: "I am enclosing a page from the Country Gentleman (February, 1949) showing the Department of Agriculture findings on the effect of weather on insects. This matter has come up in our study of *Triatoma.* The past season has been such as to confirm my ideas on this. As you may know, the early part of last year brought us a very severe drought. Day after day the fire weather forecast read, humidity critically low. Instead of beginning to gather *Triatoma* specimens in the latter part of April a very few began to appear the last of May. This stopped entirely until we had two days when the humidity rose to a point where one could smell it. During that time I was able to gather a number of specimens for the State Health Department for identification purposes in their work with the doctors. The humidity then dropped clear out of sight and with it *Triatoma* disappeared until our summer rains became normal. The *Triatoma* responded and for a few days they were crawling all over the camp. We must have had one of the night flights that you once mentioned but I had not seen. I was able to gather 47 specimens for the Atlanta Public Health Service Laboratory, and a few nymphs that hatched normally in camp. This flight must have emptied the reservoir for I caught only two adult specimens during the rest of the season." From the Parasitology Branch, Communicable Disease Center, Atlanta, Georgia, Dr. H. D. Pratt reported 12 *Triatoma longipes* and 3 *Triatomarubidauhleri* in their collection from this series of specimens.

During December of 1946, the writer collected in the foothills of the Patagonia Mountains where these insects were taken (Wood, 1949, Am. Jour. Trop. Med., *29,* 43–55). Extensive search revealed few above ground, stick-houses of the wood rat where *Triatoma* can usually be found. However, there were numerous white-footed mice and ground squirrels about and signs of mammal abundance everywhere. Besides, the terrain favors a down-canyon flight as observed at the Alvarado Mine by Wood (1943, Am. Jour. Trop. Med., *23,* 315–320) and reported from Argentina by Mazza (1936, M.E.P.R.A. No. 27, 3–48). Thus, it is assumed that these insects came from rodent burrows, or possibly bat caves, although no one has yet found the natural breeding site of *Triatoma longipes.*—Sherwin F. Wood, *Life Sciences Department, Los Angeles City College, Los Angeles 27, California.*

A SAFE METHOD OF HANDLING MOSQUITOES FOR VIRUS TRANSMISSION EXPERIMENTS

This report describes a method of handling mosquitoes, developed in an attempt to eliminate unnecessary transferring of specimens used in virus transmission studies. The procedure was found to prevent injury to the mosquitoes and minimize the hazard of accidental virus infection to the technician. Although it has been tested only with *Culex quinquefasciatus* thus far, it probably would be suitable for several other species as well.

Female pupae, in lots of any number up to 100, are separated from males on the basis of their larger size, as practiced by Kartman (personal communication, 1949). Each lot is put into a cylindrical, pint-sized, ice cream carton, the bottom of which has been replaced with paraffin-treated bobbinet, secured by the cardboard ring from the carton cover. The carton is then set into a pan containing about an inch of water, allowing the pupae to swim freely. Bobbinet is then similarly fastened on top of the carton, so that emerging mosquitoes are retained. For easy access with an aspirator later, a $\frac{3}{8}$-inch hole should be cut in the side of the carton with a cork borer and plugged with a cork.

After emergence, the number of live adult specimens is determined by counting the dead pupae and drowned adults, and subtracting their number from that of the pupae originally placed in the carton. The cast pupal skins soon dry on the bottom and cause no inconvenience. A pad

of moist cotton about 2 inches in diameter, kept at all times on the top bobbinet, provides adequate moisture to prevent desiccation of the specimens. A soaked raisin placed under the pad two nights each week serves as food.

In experiments conducted thus far, normal and infected baby chicks, 5 to 10 days old, have been used for feeding the mosquitoes. To prepare a chick for feeding, it is suspended in a 6- × 10-inch piece of muslin with an oval hole cut in the center through which the plucked breast protrudes. A liner consisting of a layer of absorbent cotton may be employed on the inner surface of the muslin to keep the chick warm and to absorb cloacal discharges. The free ends of the muslin are secured between two 6-inch pieces of lath pressed tightly together with a clamp attached to a ring stand. The carton containing the mosquitoes is placed on the base of the ring stand and the chick lowered until the bare breast is in close apposition to the bobbinet. If the chick is left overnight in this manner, an initial feeding of over 90 per cent usually is obtained.

With a curved aspirator inserted through the side-hole, it is relatively simple to remove and count the few unfed females and accidentally retained males. This number is subtracted from the total adult count to determine the number of engorged females. For removing these unfed specimens, it is convenient to work with arms inside a safety cage 15 inches wide, 15 inches high, and 20 inches deep, screened in front and on top, with a sleeve on each side and an adjustable drop-light overhead. To improve visibility, the front screen is painted black and the remainder of the cage white. The aspirated, unfed specimens are blown into another bobbinet-covered carton inside the safety cage. Should an infected mosquito escape from a carton during transferring, it can be caught or destroyed easily.

The carton of engorged specimens is retained until needed for refeeding or other purposes. Moist cotton pads and soaked raisins are made available as before. If the specimens are to be refed on another chick after digestion of the first blood meal, they may be allowed to do so without ovipositing, or oviposition can be induced by placing the carton in a shallow pan of water overnight. A considerable number of specimens usually is drowned during oviposition, due to crowding, unless relatively few are in the carton. Lots of more than 50 specimens usually suffer a rather high mortality.

If it is desired to retain the egg rafts, they can be removed with a wet pipe-cleaner inserted through the side-hole while the carton rests in enough water to keep the rafts afloat. They adhere to the pipe-cleaner and are withdrawn from the carton without escape of adult specimens if a finger is used to guard the hole.

C. quinquefasciatus has been kept alive in these containers for more than 60 days in a room with a relative humidity of 70–80% and temperature of 74°–83° F. The occurrence of mold growth on the cartons is an indication that they have been kept too moist. Under favorable conditions, they will remain reasonably clean for over 30 days.—Roy W. Chamberlain and Robert K. Sikes, *Communicable Disease Center, Virus and Rickettsia Branch, Montgomery, Ala. Public Health Service, Federal Security Agency, Atlanta, Ga.*

INFECTION WITH *ISOSPORA HOMINIS:* REPORT OF TWO CASES

During and since the war a number of reports of *Isospora hominis* infection have appeared in the literature. From these it would appear that this parasite, while extremely rare in some localities, may at least under certain circumstances be not uncommon in others. In this connection Barksdale and Routh (1948, Am. Jour. Trop. Med. *28:* 639–644) in reporting a total of 50 cases observed in the Southwest Pacific among American personnel during the war, noted that of 33 cases among 2000 troops on Luzon, 16 were found in the course of examining 270 men from one regiment. On the other hand they state that no cases of this infection were found in over 17,000 stools examined on the island of Honshu, Japan. Likewise in the examination of a total of 2524 Naval and Marine Corps personnel Markell (1945, U. S. Nav. Med. Bull. *44:* 65–68) and Markell, Mullinger and Schneider (1947, Am. Jour. Trop. Med. *27:* 63–65) found only 7 cases of *Isospora* infection, while an additional 5 were encountered in the examination of 103 civilian repatriates from Japanese internment camps on Luzon. That infection with this parasite can, at least in some patients, give rise to symptoms is now generally recognized. Clinical findings are well summarized by Barksdale and Routh (loc. cit.), while Matsubayashi and Nozawa (1948, Am. Jour. Trop. Med. *28:* 633–637) give interesting confirmation from volunteer infections.

In the past year, two cases of *Isospora hominis* infection have been diagnosed at Stanford University Hospitals. These patients both presented definite signs or symptoms of their infection, and in addition were noteworthy in that in neither case were any complete oöcysts recovered, the diagnosis being made in each instance from sporocysts, containing sporozoites, found free in the feces. (These sporocysts were carefully compared with those in mature oöcysts from a known case of *Isospora* infection.)

The first case (J.T.) was that of a 5 year old white girl, a native of San Francisco, who entered the hospital on 11–30–48 for medical observation, with a tentative diagnosis of pulmonary tuberculosis, and x-ray evidence of an enlarged heart. Aside from a history of frequent colds, fever, cough and easy fatiguability for the past 1½ years, and no weight gain for 4 months, she had had no symptoms prior to the night before entry, when she became nauseated and vomited. The next morning before entering the hospital she had 3 loose bowel movements; these did not continue after entry and in fact she became somewhat constipated. A blood count upon entry showed 7,360 white cells, with 24% neutrophils, 30% eosinophils, 40% lymphocytes and 6% monocytes. On 12–1–48 the eosinophil count was 20%. A stool specimen was obtained on 12–2–48, which showed a few sporocysts of *Isospora hominis*, as did subsequent stools for the next 3–4 days. On 12–7–48 the eosinophil count was 9%, and upon 12–8–48 had dropped to 3%. Stool examinations subsequent to this date were negative. No other basis could be found for this eosinophilia, and it was assumed to be caused by the *Isospora* infection. The relationship of this patient's brief episode of gastrointestinal disturbance to the infection is problematical.

The second patient, a 45 year old physician (Dr. M. G.), had been on a fishing trip in the Central Valley of California when on 8–21–49 he noted the sudden onset of intermittent cramp-like abdominal pain, accompanied by some nausea. He had one bowel movement at the time his symptoms first appeared, but thereafter was unable to move his bowels. These symptoms persisted intermittently, and on 8–24–49 he entered Stanford Hospital. As he had had an appendectomy in 1941, at which time multiple adhesions were found and freed, it was considered that intestinal obstruction was most likely, and a series of x-ray examinations was performed. No evidence of intestinal obstruction, or other pathological conditions, were noted. At the time of entry his physical examination was essentially negative, he had no fever, and a blood count and urinalysis were not remarkable. On 8–25–49 a stool specimen obtained by enema was found to contain sporocysts of *Isospora*. His pain and nausea continued intermittently for the next four days, although a stool examination two days later was negative. He was discharged on 8–30–49, and no more specimens were obtained.

In both of these cases the patient was constipated, and rupture of the oöcysts may have taken place because of abnormally long retention in the bowel. If this phenomenon is at all common, it may explain to some degree the apparent rarity of this parasite, which while difficult enough to detect in the feces in the oöcyst stage, is much more so when only sporocysts are to be found. —Edward K. Markell, *Stanford University School of Medicine.*

A STAIN FOR MALARIAL OOCYSTS IN TEMPORARY PREPARATIONS

So far as can be determined there have been no staining procedures recommended for malarial oocysts in temporary preparations such as are made in investigations requiring the counting of these structures. An exception, perhaps, is the procedure of Russell and Mohan (1939, J. Parasit. 25: 278–279) but this technique requires pretreatment of the mosquitoes by feeding them solutions of eosin and sugar before dissection.

During studies requiring the counting of oocysts of *Plasmodium gallinaceum* in *Aedes aegypti* it was discovered that counting could be made more rapid and almost certainly more accurate by the use of a two per cent solution of the disodium salt of 2,7-dibrom-4-hydroxymercurifluorescein (trade name Mercurochrome) which is available at any drug store.

Guts were extracted from the mosquitoes by usual methods and placed upon a microscope slide in a drop of physiological saline. To this was added a small drop of the dye solution and the preparation was allowed to stand for a few minutes after which a coverslip was placed upon the drop and examination carried out. The proportion of dye solution could vary considerably without affecting the staining and there was some latitude in time of staining. In practice, several guts were usually placed in each drop of saline and if it was inconvenient to interpret the dissections immediately after staining was complete the guts were transferred to saline without dye and kept there for the necessary time

Guts stained in this manner presented oocysts stained more or less deeply red without marked staining of the mosquito tissue. The mercurochrome was compared with eosin similarly applied and found superior in that the eosin more often stained the gut tissue. There was some variation in the intensity of staining related to the age of the oocysts but the method was found satisfactory from 4 to 9 days after feeding of the mosquitoes with subsequent incubation at 75 to 80° F.

The stain was found useful in reducing the fatigue caused by interpreting dissections and made it possible to count oocysts under lower magnifications than would have been necessary had the oocysts not been stained. In practice, about 150 magnifications were used (16 mm. objective with 15× oculars). The greatest value of the stain is undoubtedly in this feature as at high-dry magnifications the advantages of the staining were not so marked.—Don E. Eyles, *Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, and Microbiological Institute of the National Institutes of Health, Public Health Service.*

AN ADDITIONAL CASE OF *TRICHOSTRONGYLUS* INFECTION WITH A PROBABLE MODE OF TRANSMISSION

Since the report on a case of *Trichostrongylus* infection by Tsuchiya and Reller (J. Lab. & Clin. Med., 1945, 30: 262), another case was recently uncovered, this time in a native born Missourian.

The patient, a housewife, presented herself primarily for a general check-up at Barnes Hospital. As far as her physical condition goes, there is nothing important to note. However, she eats all sorts of raw foods, including fruits and vegetables. Moreover, she frequently visits her parents who live in the country. The parents own some sheep and there are some grazing animals in the area, but she does not remember the presence of any goats. Her children had a pet lamb a few years ago. The extent of her travel is negligible. She walks barefooted whenever she goes swimming.

Physical examinations were negative. Laboratory examinations revealed red blood cell count, 4,800,000 per cubic millimeter; white blood count 4,700; hemoglobin 84%. Differential count was normal with 4% eosinophiles.

Stool examination showed a few ova of *Trichostrongylus* sp., detectable only by the use of Willis' brine flotation technique. By the use of feces-charcoal culture, a few larvae, both the first and third stages, presumably of *Trichostrongylus colubriformis* were obtained. The detailed description of these larvae will be presented later. Attempts to recover adult worms, for the purpose of definitely identifying the species, following chemotherapy did not materialize as was expected. Differential morphology of ova of the parasites from those of others, particularly of hookworms, as well as from ova of plant parasite *Heterodera radicicola,* has been reported previously.

Her habit of eating raw foods, coupled with frequent visits to the country where ruminants are present, strongly suggests that, in this instance, the source of the infection may be traced to the third stage larvae accidentally ingested by the patient. It is possible also but less likely that the infection may be due to her habit of walking barefooted. It is reasonable to assume, therefore, that individuals having similar habits and exposure may become infected with the parasites. Furthermore, Mönnig stated that the larvae of the parasites are resistant to outer environmental conditions. The above assumption is further supported by the fact that cross infection between man and these animals is possible, as exemplified in the experimental study of Lie Kian Joe, 1949 (J. Parasit. 33: 359). Ransom's 1916 prediction (New Orleans M. &. S. J. 1: 294) that the parasites may be found in man in continental United States is now becoming a reality.

The recent report of Evander and Doyle, 1948 (J. Lab. & Clin. Med. 33: 869), as well as ours, amply stress the importance of calling attention to the necessity of very careful examinations of stools, not only in localities where endemicity of hookworm infection exists, but also in areas where ruminants are in close association with man.

The administration of tetrachlorethylene and, a few weeks later, the therapy of gentian violet medicinal were apparently ineffective. Repeated examination of stools still revealed a very few ova of the parasite after each course of the above treatment. Inaccurate diagnosis, therefore, may result in erroneous evaluation of anti-hookworm therapy.—H. Tsuchiya and Louis F. Aitken, *Washington University School of Medicine, St. Louis, Mo.*

PARASITES OF THE WILD TURKEY, *MELEAGRIS GALLOPAVO INTERMEDIA* SINNET, FROM THE WICHITA MOUNTAINS WILDLIFE REFUGE

Through the courtesy of Mr. Frank B. McMurry, formerly biologist at the Wichita Mountains Wildlife Refuge, the authors have had the opportunity to study a collection of parasites from the wild turkey, *Meleagris gallopavo intermedia* Sinnet, which is maintained in the wild state in that refuge. Mr. McMurry made the collections from 1938 to 1941 while making other biological observations on the flock.

Forty-seven hosts were examined and parasites were fixed and preserved in 70% alcohol. Since the collections were made incidental to other observations, it is not certain that all parasites were taken. It is felt, however, that this is as complete a collection as can be had from this area. In view of the fact that the wild turkey flock in the refuge is not large enough to allow for sacrificing other specimens, the report is given without attempts to supplement the McMurry Collection.

Of the forty-seven hosts examined, twenty-eight were infected with the tapeworm *Metrolias-thes lucida* Ransom, 1900. The degree of infection ranged from single specimens to as many as 50 adult worms.

Three hosts were infected by the trematode *Echinoparyphium recurvatum* (von Linstow, 1873) Lühe, 1909. This species of parasite is best known from wild ducks and is herein re-

ported for the second time in the turkey. It has been previously reported from California turkeys by Annereaux (1940; Jour. Amer. Vet. Med. Assn. 96: 62–64). His was the first record of it in this host and extended its range to North America for the first time. Other hosts which are known to harbor it are chickens and grebes, and it has been taken in Japan, Formosa and the Philippine Islands.

Five specimens of the trematode *Zygocotyle lunata* (Diesing, 1836) Stunkard, 1916, were recovered from one host. It has been previously reported in the domestic duck and goose, the curlew, ox, and experimentally from chickens in Mexico. The life history and bionomics of the species with laboratory infections of snails, ducks, rats and the sheep were described by Willey (1941; Zoologica, New York Zool. Soc. 26: 65–92). To the authors' knowledge this is the first record of it in the turkey.—J. Teague Self and J. Louis Boüchard, *Department of Zoological Sciences, University of Oklahoma.*

TROGLOTREMA SALMINCOLA IN MINK

Available references to the occurrence of *Troglotrema salmincola* (Chapin, 1926; N. Amer. Vet. 7: 36–37) in wild and ranch-raised mink are somewhat indefinite. Donham, Simms and Miller (1926; J. Amer. Vet. Med. Assoc. 68: 701–715) obtained immature flukes from one mink. Cram (1926; N. Amer. Vet. 7: 42–43) fed fish containing the metacercaria to one mink and recovered flukes which had undergone only partial development. Simms, Donham, Shaw and McCapes (1931; J. Amer. Vet. Med. Assoc. 78: 181–195) reported that mink have been found to be infected naturally.

It has long been known that consumption, by dogs and other susceptible animals, of fish (family Salmonidae) infected with the metacercaria of *T. salmincola* is often followed by a severe and frequently fatal infection, of uncertain etiology, called salmon poisoning. Earlier references to the resistance of mink to this disease have been rather vague and lacking in experimental evidence. Cordy and Gorham (1950; Amer. J. Path. 26: 617–637) discovered the elementary bodies of the etiological agent, which appear to be a Rickettsia transmitted by the trematode. These authors inoculated four mink with suspensions of spleen and lymph nodes from an infected fox, without any ill effects. At the same time, dogs and foxes were readily infected with the same inocula. Further presumptive evidence that mink are resistant to salmon poisoning is shown by an outbreak of the disease on a fur farm in Hood River, Oregon, as reported by the above authors. The majority of the 75 foxes on the ranch became sick, and 25 died prior to diagnosis of salmon poisoning. Treatment with sulfonamide soon stopped the losses. None of the mink on the ranch showed any signs of illness during this time, although receiving food from the same mixtures as were fed to the foxes.

During January and February 1949, a number of Aleutian mink carcasses were submitted by an Oregon fur farm to the Fur Animal Disease Research Laboratory, Pullman, Washington for autopsy. Four of 13 of these mink examined for intestinal parasites were found to be infected with a small trematode, which was identified as *T. salmincola.* The mink carcasses were unsuitable for the preparation of satisfactory histological sections. Although the cause of death was not determined, the history and symptoms of the cases gave no reason to suspect that the trematode or the Rickettsia of salmon poisoning were involved. The owner of the mink ranch reported that he had started feeding frozen salmon the preceding fall. Since prolonged freezing kills the metacercaria of *T. salmincola,* it seems likely that some of the fish had been frozen only a short time before being fed to the mink, or that some fresh fish had been included in the ration. On request, fecal specimens from 10 other mink on the same ranch were preserved and sent to this laboratory for examination. One of these specimens contained ova identical to those of *T. salmincola.* No other parasite ova were found.—Gracia A. Baker, *Fur Animal Disease Research Laboratory, U. S. Dept. of Agriculture, Bureau of Animal Industry,* in cooperation with the *Division of Veterinary Science, Washington Agricultural Experiment Stations, State College of Washington, Pullman.*

THE ARTIFICIAL HATCHING OF EMBRYONATED *ASCARIDIA GALLI* OVA

The process involved in the hatching of *Ascaridia galli* ova within the host is still experimentally unsolved. Ackert (1931, Parasitology, 23: 360–379) and the writer have observed that embryonated ova, although hatching normally in the fowl duodenum, may occasionally hatch in water cultures. Mature embryos have a distinct oral prominence, but its use as a hatching spine has not been shown (Ackert, ibid.). Dorman (1928, Tr. Am. Micr. Soc., 47: 379–413) succeeded in hatching a few *Heterakis papillosa* ova with a sulphuric acid-saline solution. All attempts at hatching *Ascaridia galli* ova with the sulphuric acid-saline solution, artificial digestive juices, and temperatures up to 37° C. were negative (Ackert, 1931, Parasitology, 23: 360–379).

It has been observed by the present investigator, while making egg cultures, that ascarid ova have a tough shell which grates under the spatula when crushed. Christensen, *et al.* (1942, Tr. Am. Micr. Soc., 61 : 191–205) stated that the egg of *Ascaridia galli* had three layers, and that the hard layer was composed of chitin. Baker, *et al.* (1929, Poultry Sci., 8 : 59–76) suggested that pressure may be a hatching factor.

Since all attempts at hatching *Ascaridia galli* ova by digestion have failed and pressure has resulted in the liberation of only a few larvae, it was decided to study the use of abrasives in the artificial hatching of *Ascaridia ova.*

The *Ascaridia galli* ova were incubated and collected according to the procedures of Riedel (1950, Tr. Am. Micr. Soc., in press). Silicon carbide, a lapidary product, was used as the abrasive because of its remarkable cutting properties and its availability in known grit sizes.

The embryonated ova were placed into a membrane made from the small end of a toy, rubber balloon, and a water paste of silicon carbide was added. The opening of the membrane was closed by a series of twists, and the mass was rolled between the fingers. After a short period of grinding, the mass was flushed into a glass cylinder, shaken and set aside for a few moments. When the abrasive had settled, the water was poured into centrifuge tubes, and the larvae and ova were concentrated by centrification.

Abrasives of grit sizes ranging from 100–200 were the most satisfactory. The abrasive action of large grits (50) were rather severe on the free larvae, while grit sizes of 400 and 600 had a tendency to remain in suspension too long during the sedimentation step.

It was rather easy to obtain from 15–25 percent hatchability. Results above this depended largely upon the consistency of the silicon carbide mass, and the length of time the ova were ground.

Attempts made to separate the liberated larvae from the unhatched ova by the Baermann technic were not very successful.—BERNARD B. RIEDEL, *Disease Research, Poultry Department, University of Georgia, Athens, Georgia.*